Studies of Lung Function

A thesis submitted for the degree of
Doctor of Science
of the
University of Edinburgh

by

Andrew Davies

DECLARATION

This work has not been submitted for any other degree or diploma and each piece of work included is either my own or in those pieces involving others I have made a significant contribution in terms of initiating the work, leading the research and in writing up the material.

Andrew Davies
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THE UNIVERSITY OF EDINBURGH

ABSTRACT OF SUBMISSION FOR HIGHER DEGREE

(Postgraduate Regulations1.1.3)

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This thesis outlines the candidate's contribution to the study of Respiratory Physiology in two main areas

1. The effect of lung morphometry on lung function and
2. Reflex control of pattern of breathing.

The work that makes up this thesis is laid out in largely chronological order describing the evolution of the investigations.

The effect of bronchial tree structure on function was investigated using a number of new techniques developed by the author. These include a method of modelling the bronchial tree to previously unobtained detail in the form of a hollow cast. This enabled gas transit times to airways of 2-3mm diameter to be measured and the contribution made by architecture, tissue compliance and the gradient of pleural pressure to the distribution of ventilation to be apportioned. This was the first time transit times to individual airways had been measured. Using these techniques the effect of bronchial tree structure on the phenomenon of separation of gas mixtures into their components during breathing, and the effect of the beating heart on the mixing of gases during breathing was quantified.

The author's contributions to the investigation of neural control of breathing follow. A fortuitous observation that SO_2 blocks pulmonary stretch receptors (PSR) in rabbits, which took place while developing an animal model of bronchitis, lead to the observation of a non PSR mechanism determining inspiratory time (t_I). Investigation of the action of rapidly adapting pulmonary receptors (RAR) using SO_2 confirmed their role in provoking sighs or augmented

breaths and demonstrated that they terminated expiratory duration (t_E) with a constant latency. A consistent effect of RARs on inspiration proved elusive until it was discovered that after provoking an augmented breath t_I is refractory to the direct effects of RAR activity for about 2 minutes. This observation lead to the development of a theoretical model of control of t_I via a central linking. This explained our observation of a non-PSR effect restricting t_I after SO_2 block. Further investigations confirmed a role for RAR in control of breathing in conscious dogs. The action of RAR in initiating inspiration was demonstrated using PSR block. The same technique was used to elucidate the role played by PSR in shifts in functional residual capacity during changes in posture. An interesting observation made at this time is that although cough is primarily associated with RAR activity it can not be triggered from the lungs. The results of experiments demonstrating a similar role for RAR in conscious animals are presented.

The influence of high frequency ventilation, on pulmonary receptors, the reflexes they produce and on the non-Newtonian properties of bronchial mucus is described.

The way in which different species control their very different frequencies of breathing is included and the way pulmonary receptor activity is changed in some models of lung disease. The effects of modern anaesthetics on receptor activity and the effect of acupuncture as a respiratory stimulant are reported.

The results of some investigations of human movement and tremor are presented.

The candidates contributions to books and books published are described.

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Introduction

This Thesis outlines the published aspects of my work in Respiratory Physiology which in research and teaching has occupied almost the whole of my working life. I have dealt with my contributions in a chronological order because, I hope, my investigations have gone forward in a logical order, each piece of work posing the next question, with a few digressions into subjects that have taken my fancy.
This enables me to conveniently lay out my work in three main Sections 1) that related to Lung Morphometry. 2) that related to Control of breathing and 3) a short Section on my interests in Human Movement together with Technical contributions. These Sections are divided into Topics in which I outline in one or two pages the rationale for carrying out that work and the results obtained. These outlines do not attempt to describe methods or results in detail but engage the reader in the principles which interested your Author in the Topic.
References to published works are found either in "Theses References,"(Section 5) referring to the work of your Author, or the "General References",(Section 6) for other workers. Copies of the published work outlined under each of the topics, in its original form, is found in Section 6- "Publications". This section includes Book Chapters, the Second Edition of "Respiratory Physiology" and an outline of the books "Human Physiology" and "Respiratory System" in production by the Publishers Harcourt.
I still have an amount of material on receptor activity in models of human lung disease which remains to be published since retiring from the University of Edinburgh in 1996. That work will be coming to press in good time.
An introduction to this thesis would be incomplete without an expression of thanks to my friends and colleagues in whose laboratories I have worked and whose dedication to Physiology and kindness to your Author has enabled him to indulge his interest in Respiratory Physiology.

Section 1. SUMMARY OF STUDIES OF BRONCHIAL TREE MORPHOLOGY

Section 1.1 Introduction to the Effects of Morphology

The work described in the first section of this Thesis describes in chronological order my contributions to the understanding of the factors which affect distribution of ventilation in the mammalian lung. The historical background and state of knowledge of the effects of structure of the lung on this function at the time this work was started in 1970 is first briefly described.

AIRFLOW IN MAMMALIAN LUNGS

The pre-scientific era of the history of respiration has its beginning when man became aware that the act of breathing was the very essence of life. The awareness of this vital function, coupled with an inability to comprehend the mechanisms involved elevated breathing to the status of a supernatural agency. The idea of the supernatural quality of air and breathing was absorbed later by Greek medicine and incorporated in the four elements that comprised life; earth, air, fire and water. Even Hippocrates who made significant contributions to the study of pulmonary disease based his theory of medicine on this doctrine. It was Erasisratus (250 B.C.) of Alexandria who made the fundamental contribution to respiratory physiology that respiration depends on the passage of air into the lungs. He then fallaciously concluded that the air then passed via the left side of the heart to diffuse through the arteries. The compilation and perpetuation of Greek and Alexandrian medicine became the task of the indefatigable Galen (130-200A.D.)

Galen postulated the blood in the left ventricle mixed with inspired air, generating fuliginous sooty vapours which were removed with exhalation. Galen's anatomical

and physiological views remained in authority for nearly fourteen centuries.

The eminent Renaissance artists conspicuously furthered the advancement of scientific anatomy. Prominent was Leonardo Da Vinci (1452-1519) who described and drew the diaphragm, the pleural cavities and the lungs.
He also remarked on the dilation and distension of the lung substance. Leonardo made wax casts of the human bronchial tree, and with a typical leap of intuition observed that their structure would affect function. None of his casts have survived and so we are not able to say if it was some defect in his casting process that made him come to the false conclusion that the total cross sectional area of the bronchial tree is constant at all levels, the decrease in airway diameter as one penetrates the lung, he said, being exactly compensated for by the increase in their number. In 1543 Andreas Vesalius described how an open-chested animal could be kept alive by aerating its lungs with a bellows; he also noted that a failing heart could be revived by this process. The minutes of the Royal Society record a similar experiment by Robert Hook(1635-1702) who blew air through the lungs of a dog with an open thorax after pricking holes in the pleura, thus proving that a constant supply of fresh air was essential for life, and that the mechanical movements of the lungs and chest were of secondary importance. Present day concepts of air flow in the lungs were initiated by Einthoven (1892). It was Einthoven who originally suggested that compression of the intrathoracic airways took place on forced expiration. His theories were later confirmed by Damen (1951) although the degree and site of compression still provoke argument. Roher (1915) in a physico-mathematical approach to the problem of the distribution of airflow in the lungs based his calculations on measurements made by himself, Danders (1818-1889) and others. His work, which covered the major points of fundamental importance in respiratory mechanics was marred by an arithmetical error which lead him to overestimate by a factor of 10 the flow in the tracheobronchial tree at which turbulence takes place. He therefore calculated that flow was laminar in the human tracheobronchial tree at almost all respiratory rates and that the majority of airways resistance resided in the small bronchioles. It was not until Gansler (1952) began to

investigate pressure/flow relationships in endotracheal tubes that it was realised that turbulence in the trachea takes place at much lower flow rates than previously supposed. The calculations of Webel (1963) made from measurements of solid casts and the studies of Macklem (Macklem et al 1963; Macklem and Wilson 1965; Macklem and Mead 1967) indicate the majority of airways resistance to be situated in the larger airways. Other factors such as the force of gravity, branching angle of airways and the fluid lining of the airways have now been recognised as influencing the mechanical properties of the lung. The work of Webel, based on measurements of resin casts of the airways of the lung, emphasises the primacy of the architecture of the bronchial tree in determining distribution of inhaled gas. On this the effects of other variables, mechanical properties and gravity for example, are superimposed.

No part of the human or mammalian body shows more important anatomical variations than the lung. Today the variations of the human lung have been recorded in detail, and the basic anatomical patterns which underlie them established. Yet sixty years ago (1940) R.C.Brock in "The Anatomy of the Bronchial Tree" states " as soon as the lobar branch enters the lung substance and begins to divide it is virtually uncharted country". He adds "Only by the use of bronchial casts and of specimens in which the segments have been injected can the full story be learned and depicted".

The history of the evolution of bronchial casts as a scientific tool is described by Davies (1975) . Various investigators have proposed models of the tree based on morphometric studies of excised lungs or solid casts of the bronchial tree, These theoretical models were considered useful in making calculations appropriate to a particular investigation (Rohrer 1915; Findeison 1935: Hilding 1948; Landahl 1950).

Ross (1957) was the first to point out that these models have a common limitation that average values of lengths and diameters of bronchi of the various orders of branching are used in their construction. Implicit in this type of construction is the inference that all alveoli are ventilated through identical bronchial pathways, each having the same number of component bronchi of identical calibre and equal

length. Solid resin casts of the bronchial tree were exhaustively exploited by Weibel (1963) in his morphometric study of the human lung. West and Hugh-Jones (1959) prepared hollow resin casts of the human trachea and large bronchi and passed water and air through them to determine the point of onset of turbulence. This approach promised an experimental method of testing the theories based on mathematical models, and was the path I determined to follow to investigate the effect of bronchial tree structure on function.

Consequences of Airway Morphology

Until the work of Ross (1957) the part played by the asymmetry of the bronchial tree in the determination of lung function had received little attention. Ross stressed the importance of this asymmetry in the production of inequality of ventilation in the dogs lung. Weibel (1963) in his analysis of the human bronchial tree used a resin cast of the airways which he measured completely to generation five and incompletely to generation ten. In predicting the structure of the smaller airways he originally assumed regular dichotomy which automatically excluded inhomogeneity due to asymmetrical bronchial anatomy. This was corrected in his Model B analysis.

To include the effects of the asymmetry of the bronchial tree Horsfield and Cumming (1968a) carried out morphometric studies on a resin cast of the bronchial tree and adopted a novel system of nomenclature that ordered the airways from the smallest to the trachea; rather than the conventional system of starting with the trachea and working to the periphery. This method of ordering the system led them to consider the bronchial tree as a dichotomous branching system "with a few pieces missing". In a subsequent paper (Horsfield and Cumming 1968b) they considered the functional consequences of their findings. In anticipation of the calculations in this paper Cumming (1967) pointed out that "if diffusion (in the terminal airways) is complete, and therefore makes no further contribution to the inefficiency of gas mixing, the total inefficiency will be determined by those factors affecting transit times". Transit time being the time takes by a front of gas to pass from the carina to any defined point in the bronchial

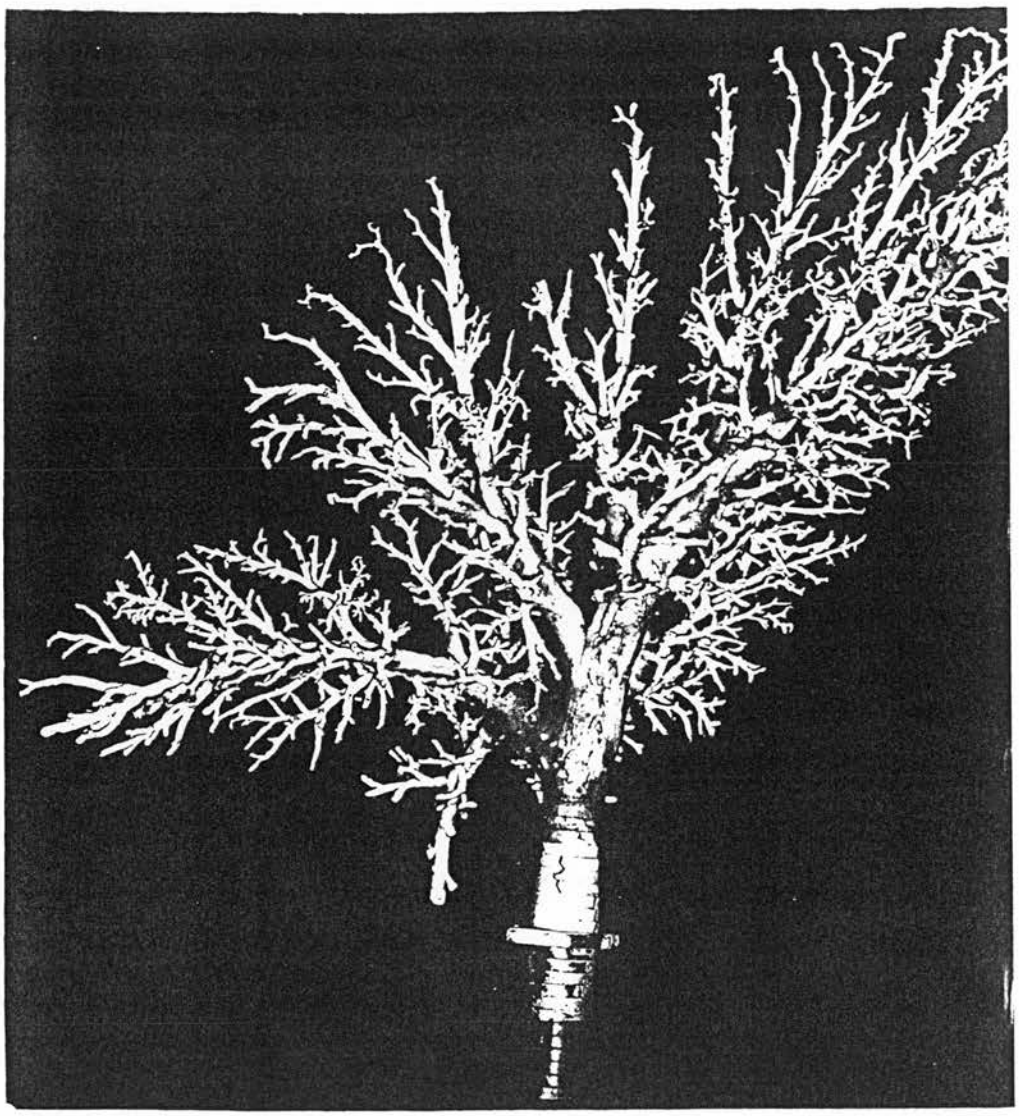

Fig.1. Hollow Cast of Pig Lung.

tree. Wagner et al (1969) measured the time taken by carbon monoxide injected at the larynx to appear in the capillary blood of dogs. Their observations were restricted to a single area on the lung's surface and so they could not estimate the dispersion of transit times, if any, that existed in the lung.

The work described in this section of this thesis consisted of developing a method of producing hollow casts of the bronchial tree in previously unobtainable detail, and an instrument capable of detecting the arrival of a gas front in small airways. At this time the only instruments capable of the speed and sample rate required were mass-spectrometers which were too bulky, unreliable and expensive. Using the detection system I developed transit times were measured in hollow casts of the bronchial tree, in excised lungs and in excised lungs subjected to a gradient of pleural pressure. In this way the factors affecting transit times could be studied in isolation, and their respective influence noted. These measurements were used to test the hypothesis, promoted by morphometric studies, that the asymmetrical structure of the bronchial tree produces the basic form of uneven ventilation, upon which the effects of compliance and a gradient of pleural pressure are superimposed.

The development of the detailed hollow cast was also fundamental to the direct investigation of the effects of structure on the distribution of airways resistance and the separation of gas mixtures as they pass into or out of the lung and is described in the next section.

Section 1.2 Methods of Casting the Bronchial Tree

Hollow silver casts representing in previously unobtainable detail the architecture of the bronchial tree provided one of the main instruments in the investigation of the effect of bronchial tree architecture on function. It is therefore worthwhile reviewing previous attempts at the production of casts of the airways of human lungs. It should be noted however that the majority of previous casts were solid, and used for demonstration or morphological purposes only.

HOLLOW CASTS

A picture of one of my casts is shown opposite

In 1689 Govert Bidloo recorded the preparation of a metal cast of the human bronchial tree. He claimed this was produced by the injection of molten bismuth. However the melting point of bismuth (271^0C) is far too high for this to be possible. Probably an amalgam of bismuth and mercury was used. These amalgams are no stronger than wax, which may explain why none of Bidloo's casts have survived.

In the same year as the production of Bidloo's casts was recorded, Ysbrondi von Dimerbroeck, Professor of Medicine and Anatomy at the University of Utrecht, published a simple woodcut of a cast of the human bronchial tree. During the next century the art of injecting with coloured wax, invented by Jan Swammerdam (1637-1680) reached the pinnacle of perfection. In 1790 Thomas Pole published the first general textbook of anatomical techniques including a section on this type of injection of the lung. Apart from his mastery of technique Pole had clear insight concerning personal qualities essential for this type of work. He says "In making casts the principle ingredients, and the first to be obtained, are time and patience, and not less so an uniform fortitude against disappointment; for it will not infrequently happen, that with the greatest care, a most promising preparation will be instantaneously destroyed by some trivial accident, when it has almost been completed". The following sound advice is given on the care of finished casts; "Preparations injected for corrosion should always be carefully handled lest the injection be incautiously broke, which in their finished state, having no support from the surrounding vessels will fall to pieces; this would be an unpleasing circumstance after everything else had been successfully concluded".

He goes on to say "These preparations require great care and much time to complete them, and when finished are of all others most liable to be demolished by trivial accidents; it is therefore expedient to defend them as much as possible from injuries, for persons who have not made them are not always satisfied with looking, but every now and then trying their strength by the finger, at the expense of destroying the most beautiful parts".

The nineteenth century was the age of the comparative anatomists and they did not

overlook the mammalian lung. The Swiss anatomist Christoph Theodor Abey in 1880, aided by somewhat distorted metal casts managed to see in the human lung a main stem bronchus extending to the base of the lung and giving off alternate ventral and dorsal branches. Abey's prestige was immense and his description of the branching of the human bronchial tree was enshrined in textbooks of anatomy for the next fifty years. Ewart (1889) challenged Abey's views and adopted the view, since universally accepted, that the branching of the lung airways is adapted to the shape of the thoracic cage and hence comparison of other mammalian lungs with human lungs can be misleading if this is not taken into account.

Since that time anatomists have recorded in detail the variations of mammalian lungs and established the basic anatomical patterns which underlie them. No resume of the use of lung casts would be complete without reference to Weibel (1963) whose monograph marked a new era in the development of quantitative anatomy and the correlation of anatomy with physiology.

Production of the Hollow Casts

The casts described above are "negatives" in that they represent the air spaces rather than the airway walls of the bronchial tree. Their use has therefore been limited to demonstration of anatomical features and pathological changes. Made with care these casts keep the fine detail of the airways as far as the bronchioles. Hollow casts for use in airflow studies have been made from these negatives by conventional casting techniques. Some, made in resin, have contained airways from the trachea to the segmental bronchi (Dekker, 1961) but all suffer from the lack of detail which makes them unsuitable for study of aerodynamics of the bronchial tree. The hollow casts used in the works described in the first part of this thesis on the other hand preserved the detail of the solid casts and were made by the method described in Timbrell.,Davies,Bevan,.*et. al.*(1970) and shown opposite Page 12

In summary a solid wax cast of the air spaces was first made using the method of Weibel and Vidone (1961) in which the lung was inflated by negative pressure in an artificial thorax. Molten paraffin wax was poured into the bronchial tree and allowed to set. The wax contained radio-opaque iodized oil and when the wax was set the lung was X-rayed to ensure satisfactory penetration .

The tissue was digested off the wax in a bath of concentrated hydrochloric acid, which took 2-3 days. The wax cast was then sprayed with a conducting layer of colloidal silver and electroplated, except for the finest ends of the airways which had been protected by a further layer of molten wax spread over the silver. Electroplating continued until the silver was 0.01 inches thick. The wax was removed from the cast by heating to 80^0 C and blowing heated air through it.

Comparison of Casts with Bronchiograms

A solid or hollow cast is obviously an artificial replica of the bronchial tree, and it remained for me to see whether its aerodynamic properties and dimensions compare satisfactorily with those of the living organ.

Weibel (1963) compared the dimensions of the plastic casts he used for his morphometric studies with bronchiograms of healthy young adults. The dimensions of the bronchial generations 0 to 4 were estimated from the bronchiograms. Diameters agreed well with those from the cast and the lengths were of the same order of magnitude. The volume of the cast from the carina to the eighth generation agreed well with that calculated from the bronchiogram. The measurement of length of the image of a bronchus in such a study, and in my own study, is rather uncertain because of the inclination of the bronchus to the plane of view, and because the origin of the bronchus can rarely be localised with certainty. In my study, when the lung had been filled with radio-opaque wax and the tissue surrounding it dissolved, it was much easier to compare the cast produced with the bronchiograms of the whole lung taken in three vertical projections. This process enabled damage to the initial wax cast to be detected, as well as incomplete filling of the limbs which could result in an unsatisfactory cast being produced. These comparisons indicated that the airways and the dimensions of the airways in the pig lung were of the same order as those found in the human.

Section 1.3 Methods of Measuring Transit Time

The observation by Cumming (1967) that "if diffusion (in the terminal airways) is complete, and therefore makes no further contribution to the inefficiency of gas mixing, the total inefficiency will be determined by those factors affecting transit

NOT CLEAR

times". (transit time being the time takes by a front of gas to pass from the carina to any defined point in the bronchial tree) makes measurement of the distribution of transit times a cardinal method of measuring total inefficiency of gas mixing in the lung.

Wagner *et al* (1969) measured the time taken by carbon monoxide injected at the larynx to appear in the capillary blood of dogs. Their observations were restricted to a single area on the lung's surface and so, although this measurement gave insight into the nature of gas flow along the pathway concerned, they could not estimate the dispersion of transit times, if any, that existed in the lung. The method used by Wagner *et al* was photometric, dependant on the spectral change of haemoglobin in capillaries serving several thousand alveoli and therefore not designed to measure gas transit time within the bronchial tree.

A method of measuring gas transit time in a branching series of tubes of diameter down to 1.5mm (casts of the bronchial tree) was developed as reported by Davies,(1971,1972). This consisted of injecting a bolus of halogenated hydrocarbon gas (dichlorodifluromethane) into the ventilating air stream. The arrival of the halogenated hydrocarbon at distal points within the system of tubes was detected by a halogen sensitive diode of the type used as a leak detector in high vacuum engineering through which a vacuum pump drew a small continuous sample of gas via a catheter implanted at the chosen point. A second diode, sampling gas near the carina in the case of hollow casts, acted as a fixed reference point. The time interval between the signals from the two diodes (after allowing for the delay due to the sampling catheters) was the transit time between the two sampling points.

In hollow casts, which were used to investigate the influence of the asymmetrical structure of the bronchial tree on transit times, samples were drawn from 1mm diameter holes drilled at 1cm intervals along pathways from the carina to the periphery. Superimposed on the effects of structure, or architecture, of the bronchial tree are the effects of differences in compliance of the respiratory regions of the lung and the effects of the gravity induced gradient of pleural pressure on these differences in compliance. The methods of measuring transit times to

airways of the same diameter and of producing a gradient of pleural pressure while making these measurements is described in Davies(1972). In brief retrograde catheters with bell shaped ends of diameter equal to the airways from which samples were required were passed down the airways and out through the plura. This is a method used by Macklem and Mead (1967) to measure pressure in airways without interfering with regional ventilation. In this way the bell shaped end lodged in an airway of the required diameter while the free end of the catheter could be attached to the halogen detector and the system ventilated as in the case of the hollow casts. This step from rigid hollow casts to ventilated excised lungs takes in the effect of differences in regional compliance of the lungs and superimposes it on the effect of the architecture of the bronchial tree. The final major factor affecting distribution of ventilation in the lung is the gradient of pleural pressure which exists over the upright human lung due to the effect of gravity. Various methods have been used to mimic this effect, the method of Zardini and West (1966) who used a foam produced by beating a 20% egg albumin solution to produce something akin to a meringue approximates most closely to the method I used. This consisted of a fluidised bed produced by blowing air through a container of polystyrene beads. The density of the beads could be pre-determined by heating them for various times and by this method a "virtual liquid" of a density 0.25 could be produced. This virtual liquid produces a pressure gradient equivalent to that found in the intrapleural pressure in the chest of an upright man.
Combining the fluidised bed with a ventilated excised lung in which transit times are being measured using the retrograde catheters enabled the final step in the series-

Hollow Casts - demonstrating the effect of architecture alone

Excised Lungs- adding the effect of regional compliance

Excised Lungs in a Fluidised Bed- adding the effect of a gradient of pleural pressure, to demonstrate how each of these components adds to the dispersion of transit times in the whole subject.

Section 1.4 Airflow and Turbulence in Casts

At the time these hollow casts were being developed there was considerable debate as to the major site of airways resistance in the lung, and even as to the nature (lamina or turbulent) of airflow in different regions of the lung. The nature of airflow was considered of importance not only because it would affect work of breathing but it would also determine the nature of the advancing gas front during inspiration and the deposition of particles in the lungs.

The first quantitative study of air flow in models of the human lung was undertaken by Rohrer (1915). He calculated that transition from laminar to turbulent flow took place in the trachea at a flow-rate of 3.5 $l.s^{-1}$. As this flow is rarely achieved during normal breathing he assumed that flow is likely to be laminar in the bronchial tree throughout the respiratory cycle. Not until Gainsler (1952) began investigating flow/pressure relationships in endotracheal tubes was it realised that Rohrer had made a dimensional error in his calculations and overestimated by a factor of ten the critical flow-rate in the trachea. Gaensler calculated the critical flow-rate for a tube of the same size as that used by Rohrer to be 0.49 $l.s^{-1}$. Quite inappropriately both sets of calculations were based on physics relating to long straight cylindrical tubes with smooth walls and ideal inlet conditions; hardly conditions existing in the tracheobronchial tree, where the airways are short and branching with rough walls and irregular cross-section. These criticisms could in part be answered by direct measurement in the biological system or an exact physical model of it. West and Hugh-Jones (1959) constructed transparent casts of the upper airways and used water as the flow medium to determine the critical flow-rate. Dekker (1961) used the same technique but including the effect of the larynx to conclude that the larynx reduced the critical flow-rate from 0.35 to 0.12 $l.s^{-1}$.

This suggests that turbulence is more widespread than previously supposed, but these studies and even those of Clarke (1969) and Schroter and Sudlow (1969)

followed airflow for no more than two generations of branching. They did however demonstrate the important fact that the disturbance of the velocity profile across the airway caused by each junction is not dissipated before the next junction is reached, and the limitations of mathematical models such as Rohrer's in which the term K_2V^2 in the equation relating pressure drop to flow

$$P= K_1V + K_2V^2$$

attempts to include this effect but does not consider it in detail.

Mathematical modelling of airflow in the lungs at this time also suffered from lack of adequate morphometric data on the airways. Even the classical work of Weibel (1963) who considered the human lung as a symmetrical dichotomously branched system; and Horsfield and Cumming (1968b) who introduced the factor of asymmetry into their calculations did not include details of airway taper, branching or surface roughness in their data.

To obtain data of pressure gradients and therefore resistance in the bronchial tree, for its own sake or for the validation of models of the system, it was clear that direct measurement in the organ itself or in a hollow cast of considerable detail was required.

While such casts are obviously a static replica of the bronchial tree they are useful in providing a detailed and exact model of the system fixed at some point in the respiratory cycle. The hollow casts used to measure transit time offered the opportunity to make such measurements.

The pressure gradient along a stream of gas flowing through a tube of non-uniform diameter may be considered to be of two components. That due to the frictional properties of the gas and the airways in which it is flowing and an inertial pressure due to the acceleration of the mass of the gas. The acceleration of the gas can be further divided into local acceleration, which is the rate of change of velocity of the gas particles with time, and convective acceleration, which is the rate of change of velocity in relation to distance travelled along the constraining tube. Convective acceleration would occur when the tube in which the gas was flowing had converging or diverging boundaries, as is the case in the bronchial tree.

The pressure drop associated with local acceleration of gas in the airways has been shown by Mead (1956) to be negligible. The pressure drop associated with convective acceleration may not be negligible. Hyatt and Wilcox (1963) estimated that it accounted for 34% of the total pressure drop in the lower airways of their subjects. Failure to consider this leads to an overestimation of expiratory frictional resistance and an underestimation of inspiratory frictional resistance. However convective acceleration pressure drop can be calculated from the formula

$$Pca = \frac{K \delta V^2}{2g}$$

Where δ is gas density , g acceleration due to gravity , V average velocity across the main bronchus, and K a constant related to the spatial integration of gas velocity over the cross-section of the trachea. The value of K is reported by Rouse (1959) to be unity for a perfectly blunt profile and 2 for a parabolic front .

For the conditions used for pressure measurements in the hollow casts it can be calculated that convective acceleration contributed less than 0.003cm H_2O or less than 3% to the lowest pressure measured.

Distribution of pressure drop in a serial system of tubes reflects the distribution of airway resistance. It is now well accepted that if total pulmonary resistance to breathing is divided into its components (Marshal and Dubois,1956; Ferris,1964) aerodynamic resistance represents an important part. The rigid casts used in the study being described in Davies (1974) isolated aerodynamic resistance from tissue resistance and enabled succeeding generations of the bronchial tree to be considered as resistances in series and parallel.

Pressure/ flow measurements in the hollow casts demonstrated

a)the variation in airway resistance of individual pathways of the casts was less than variation in pathway lengths and less than might be expected from their very different geometries (which was not quantified).

b)this small degree of variation in resistance was accounted for by the longer pathways containing a larger proportion of large diameter airway.

c)the majority of airway resistance of each pathway existed in airways larger than

2mm diameter. Beyond that point smaller airways provided a large number of parallel pathways greatly increasing the total cross section of the conducting tubes.

Section 1.5 Transit Times

Inequality of ventilation in different regions of the mammalian lung can be ascribed to two main sources. Firstly stratified inhomogeneity results from the decrease in velocity of a gas front passing down the bronchial tree due to the increasing cross-sectional area of the airways. The slowing of the advancing front continues until in the fine airways diffusion dominates bulk flow in determining forward movement of the gas molecules. A gradient of concentration along the airway results.
Regional inhomogeneity, the second type of inequality, is the result of unequal distribution of ventilation among the terminal units of the lung. This inequality may exist in terms of time (sequential ventilation) where regions filling early in inspiration receive a larger proportion of dead space gas than those filling later; or in terms of space where inspired gas has to traverse different distances from the lips to reach the respiratory surface. This means that different amounts of dead space gas will precede inspired air into different respiratory regions.
Airways resistance measurements at different levels of the bronchial tree (Macklem and Mead(1967) and Davies(1972) suggest that the resistance to flow offered by the airways has only a small influence in the distribution of gas in the lungs, and that bulk flow is largely governed by airway morphometry, the elastic distensibility of the air spaces and the differences in the distending forces acting on them.
The implication of unequal distribution of dead space gas is that the time taken by a gas front to reach the respiratory regions of the lung varies throughout the lung. Ross (1957) defined this interval as "transit time", Cumming (1967) has pointed out that if diffusion is complete the degree of uneven ventilation in the lung will be completely determined by those factors affecting this transit time. In Davies(1975) direct measurements of transit time from the carina to points within the bronchial tree of the pig lung have been made. The effect of the asymmetrical anatomy of the

bronchial tree is reported in Davies(1971) and the effect of regional differences in compliance is reported in Davies(1972).

The object of the work described in this part of this Thesis was to attempt to measure the, until then, only theoretically described variable "gas transit time" to small airways, to measure the distribution of values of transit time and to investigate the effect on that distribution of factors thought to influence the uniformity of ventilation.

Previous investigators have demonstrated that secondary motion within the gas stream of the bronchial tree (Schroter and Sudlow,1969) and diffusion within tubes of the dimensions of small airways (Crank,1957) would result in radial mixing which is forty times faster than forward flow and result in a square front of gas advancing through the airways. This square shape to the gas front means that its arrival at a point within the bronchial tree could be detected with precision .

Because Davies(1975) reports the first ever measurement of gas transit time in the mammalian bronchial tree, and because I wished to compare this index of distribution of ventilation with other indexes, almost all of which were made in man, (even though they did not partition the factors giving rise to the distribution of ventilation) I wished to select a species comparable in size and anatomy to man, for that reason pig lungs were used.

Transit time through any segment of the bronchial tree is related to the length and cross-sectional area of that segment. The curvilinear relationship between transit time and distance in both casts and excised lungs reflects the increase in cross-sectional area and the close identity of the curves obtained from both demonstrates the similarity of structure in both .

The hollow casts represent the behaviour of whole lungs in which the effect of structure of the bronchial tree on ventilation has been isolated. This effect was then compared with the effect of regional differences in compliance, if any, in the isolated lungs.

The results obtained with the hollow casts demonstrate conclusively that the asymmetrical anatomy of the bronchial tree results in a distribution of transit times, and hence uneven ventilation, throughout the lung. On this distribution the effects

of differences in regional compliance and a gradient of pleural pressure are superimposed.

Transit time to airways of a particular diameter was related to its distance from the carina. The functional consequences of this are-

(1) dead space gas will be unevenly distributed in the lung.

(2) the gasious interface between inhaled and dead space gas is established at different distances from the respiratory surface.

(3) the time which an interface spends in the respiratory region varies throughout the lung.

A major influence on these factors is the increasing cross-sectional area of the bronchial tree. This results in a rapid reduction of the linear velocity of the advancing front as it reaches the peripheral airways. This means that for a large part of the respiratory cycle the gas front is almost stationary and a very large surface area exists between it and the receding front of dead space gas. The functional consequences of these facts are emphasised by Johnson and van Liew (1974) in their demonstration of the importance of molecular diffusion in oxygen transport to the alveolar wall. The effect of these characteristics of the advancing gas front on the separation of gases of different diffusivities is described in Horsfield, Davies and Cumming,G. (1979) and Horsfield, Davies and Cumming (1980).

The slowing of the advancing front was seen in casts ventilated with a constant inspiratory flow which demonstrates it is a function of cross-sectional area rather than an effect of compliance or cyclic ventilation.

Mean transit time to airways of 0.16cm diameter, and the distribution of such transit times was essentially the same in casts and excised lungs, demonstrating that any lobar differences in compliance were not enough to affect the transit times established by the effects of bronchial anatomy.

Gravity effects on the lung must be considered in any measurement of transit time. Studies of bronchiospirometry (Koler,Young and Martin, 1959) and scanning the lungs after inhalation of ^{133}Xe (Dollfus, Milic-Emili and Bates,1967) have shown

that the distribution of ventilation at low flow rates is mainly related to regional expansion of the lung.

Lobar differences in lung compliance are found in dogs (Faridy, Kid and Milic-Emili, 1967) and it has been suggested that these interact with the gravity induced gradient of pleural pressure in man (Bake,Bjure,Grimby,Milic-Emili,1967). The fact that such a gradient affects the distribution of ventilation has been demonstrated *in vivo* (Ball,Stewart,Newsham and Bates, 1962; Bryan,Bentivoglio,Becrel,MacLeish,Zidulka and Bates, 1964; Milic-Emili,Henderson,Dolovich,Trop and Kaneko,1966) and in excised lungs (Zardini and West,1966) . The results cited in Davies(1975) suggest that the major component affecting this distribution is not differences in compliance but the gradient of pleural pressure over the upright lung.

In Davies(1975) the effect of a gradient of pleural pressure in increasing transit times to dependent regions in the normal "head up" situation is described. In the "head down" position transit times to what was normally the lower lobes were not significantly different from the values in the supine position. The relative immunity of transit times in the upper lobe to changes in the pleural pressure gradient was ascribed to the shorter vertical distances from the sample sites in this area to the carina.

This increase in transit times to dependent regions is explained in terms of effective compliance in the intact animal. Regions exposed to the most negative pressure expand first at the beginning of inspiration, causing air flow to the upper regions. Horsfield (1967) has demonstrated morphometrically that the mean airway pathway length in man is greater to the lower lobes than the upper. The effect of gravity on the upright lung will therefore be to increase the distribution of transit times that would arise from purely anatomical reasons.

The results reported in Davies,A.(1971,1972a,1972b & 1975) were obtained with unique hollow casts produced by a method described in Timbrell,.Davies,Bevan,.*et.al.*(1970). These results demonstrate -

a) The anatomy of the bronchial tree produces a fundamental distribution of ventilation in the lung on which other factors are superimposed.

b) This distribution of ventilation can be expressed as a distribution of transit times to the respiratory surface.

c) Measurement of transit time along pathways to the respiratory surface demonstrate a profound slowing of the advancing gas front.

d) The similarity between the advance of a gas front into a rigid cast and into an excised lung suggests that the differences in compliance that exist in the lung alone are not large enough to significantly affect the distribution of transit times and hence ventilation.

e) The gradient of pleural pressure over the upright lung produces significant increases in transit times to the lower lobe.

The effect of the naturally occurring gradient of pleural pressure in upright man would therefore be to accentuate the effect of bronchial tree architecture on the distribution of transit times and therefore ventilation.

Measurement of transit times to sequential points along pathways from carina to the periphery exposed a type of gas transit that involved the development of a parabolic velocity profile and radial diffusion. This type of gas movement combined with molecular diffusion in the gas exchange area of the lungs would produce differential movement of gases with different properties. The extent to which this takes place was investigated in Horsfield,.Davies and Cumming (1979) and Horsfield, Davies and Cumming(1980).

Section 1.6 Effect of Structure on Separation of Gas Mixtures

The limitation on gas exchange in the lungs, set by the limitations of diffusion in the respiratory (alveolar) regions, has been a subject of considerable debate. We have carried out a number of experiments using hollow casts to represent in great detail the airways "frozen" at some point in the respiratory cycle in which the process of diffusion can be investigated. Georg, Lassen, Mellemgaard and Vinther (1965) investigated this process by including in an inspirate three gases of differing diffusion coefficients, namely helium (He) neon (Ne) and sulphur hexafluoride (SF_6). Relatively more SF_6 appeared early in the subsequent expirate and relatively more He appeared late. This was thought to be due to the more rapidly diffusing He penetrating further into the lung and thereby lowering its concentration in the

proximal airways. Experiments by Cumming, Horsfield, Jones and Muir (1967) , Power (1969) and Hogg, Brunton, Keyger, Brown and Macklem (1972) yielded similar results.

Hogg *et al* (1972) also produced partial airway blockage of airways in excised lungs by insufflating them with beads. After this they found that both end-expiratory and residual gas contained relatively more SF_6. This they attributed to the dispersion mechanism described by Taylor (1953) who analysed mathematically and experimentally the dispersion of a solute in a solvent flowing through a tube. The dispersion results from the combined effects of a parabolic velocity profile and of radial diffusion.

Consider a long tube, initially containing pure solvent, into which flows solvent containing a diffusible solute. A parabolic velocity profile develops and this will tend to form a parabolic concentration profile at the front of the solute. In the region of this concentration profile the solvent at the centre of the tube contains more solute than does that at the periphery, so the solute diffuses radially towards the periphery thereby changing the shape of the concentration profile. This solute moves from the more rapidly flowing central part of the stream to the slower peripheral parts, so that the longitudinal dispersion of the solute is less than it would have been had not radial diffusion occurred. Taylor showed that when axial diffusion is slow enough to be disregarded the resulting dispersion is equivalent to an apparent diffusion with an apparent diffusion coefficient k where

$$k = r^2v^2 / 192D$$

r=radius of tube v= maximum velocity of flow in the centre of the tube, and D = the molecular diffusion coefficient of the solute. The coefficient k is inversely proportional to D so that the more diffusive the solute the less is the longitudinal dispersion and the shorter the concentration profile. This dispersion occurs relative to a plane at right angles to the axis of the tube which moves along the tube with velocity $v/2$. The 50% point of the concentration profile is normally situated in this plane. However, if axial diffusion is sufficiently great so that it can not be disregarded the apparent diffusion coefficient requires a correction, in which case

$$k = D + r^2v^2 /192D$$

As the ratio of axial molecular diffusion to velocity increases the concentration profile becomes flatter and approaches in shape that which would result from molecular diffusion alone. The more diffusible substance may then undergo the greater longitudinal dispersion.

The hollow casts produced by my method were used in three types of experiments to determine the part played by the conducting airways in the partial separation of gas mixtures seen during inspiration and expiration of such mixtures and whether Taylor dispersion or the physical properties of the gases used might explain the separation.

In the first type of experiment a mixture of air, SF_6 and He was blown down the cast ("inspiration") and the gas sampled just within the open end of several peripheral airways. This showed that passing through the cast caused He to be delayed relative to the SF_6, a result which could have been due to either Taylor dispersion (He is more diffusive) or to an effect dependent on viscosity(He is more viscous).

To differentiate between these two possible explanations the experiment was repeated using Argon (Ar) instead of He. Ar is is less diffusive than He but more viscous.

In the third set of experiments the direction of flow was reversed ("expiration") using the SF_6 and He mixture to see if the separating effects are due to passage through the bronchial tree during inspiration, expiration or both.

The results of these experiments were reported in Horsfield,Davies.and Cumming (1979) and showed that passing through the cast caused He to be delayed relative to the SF_6 as described above. The second set of experiments, substituting Ar for He, provided results qualitatively similar to those obtained with He but with a lesser degree of separation. Since Ar is more viscous than He the separation should have been greater if caused by differences in viscosity, which answers the question posed above.

In the third type of experiment there was no separation of SF_6 from He in the expirate. The shape of the gas fronts at the main bronchus in these experiments was

the sum of all those coming from all pathways and their resultant probably swamps any other effects. In addition the rheological mechanisms which are operating on the front during inspiration are different from those during expiration. Schroter and Sudlow (1969) showed that four secondary vortices are generated at a junction during expiration compared with only two during inspiration. These probably have a marked mixing action diminishing any separation which might have occurred due to Taylor dispersion. These facts explain the demonstration reported in Horsfield, Davies and Cumming (1979) that the separation effects seen in the whole lung are produced during inspiration.

Hogg, *et al* (1972) found that, following the inspiration of a gas mixture, more SF_6 than He entered the alveolar region of excised lungs insufflated with beads. They attributed this enhancement of the forward movement of SF_6 to the effects of Taylor dispersion. Thus SF_6 the less diffusive gas would be dispersed longitudinally more than the He and would therefore enter the alveolar region first. This phenomenon was only found after the insuflation of beads presumably because flow went through collateral channels effectively lengthening distal pathways and impeding longitudinal diffusive mixing between gas in the alveoli and the conducting airways.

Van Liew and Mazzone (1976) performed an experiment similar to ours reported in Horsfield, Davies and Cumming (1979) except that they used a simple straight tube. When a mixture of SF_6 and flowed down the tube the SF_6 appeared at the far end before the He. Although the separation in their experiment was less their results are in general agreement with ours.

Our experiments give insight into the postulated contribution made by Taylor dispersion to the increasing of alveolar concentrations of SF_6 in single inspirates. The first and most important point is that Taylor dispersion can only operate in that part of the bronchial tree modelled by our hollow casts and swept by the parabolic gas front. In such a tubular system containing the front 50% of the volume will be occupied by occupied by the central parabolic core of a gas mixture containing SF_6 and He The maximum separation that could occur would be if no SF_6 diffused radially while all the He rapidly distributed evenly across the tube. The core would

contain all the SF_6 and half the He. In practice some SF_6 would diffuse and a perfect parabolic front would never develop, In terms of the respiratory system the volume of gas mixture separated must be less than 25% of the volume of the conducting airways.

The airways where Taylor dispersion is effective can be calculated and seen to be the regions included in our casts. If L= length and r = radius of an airway u= mean velocity of air within it and D = diffusion coefficient of the gas in question. Then in airways in which

$$r^2u/\ 10DL < 1 \text{ but } ru/10D \text{ is not } << 1$$

then Taylor dispersion becomes effective.

The relevant airways in the human lung were calculated by Wilson and Lin (1970) to be Weibel's (1963) airway generations 1-12. Even with fast flow rates and gases of lower diffusivity it is unlikely that the relevant region would extend further than the terminal bronchioles. Taking 70 ml as the volume from carina to "lobular" branches and 30ml for the volume of the remaining three orders down to the terminal bronchioles this sets 100 ml as the upper limit of the volume of airways involved. Taking our previous calculation of separation as 25% maximum the very most gas that would be separated is 25ml. In our experiment the separation, adjusted to represent two lungs was 37ml reflecting the exclusion of perturbing factors in the hollow casts.

The findings of these experiments with hollow casts explain the findings of other workers that separation of inhaled foreign gases takes place in the lungs in terms of Taylor dispersion. These gases have diffusion coefficients which vary widely. On the basis of our findings it is unlikely that Taylor dispersion contributes to the generation of the slope of the alveolar plateau, as has been suggested, a conclusion reached by Chang (1976) on the basis of theoretical calculations.

Cardiac Mixing of Inspired Air

The slowing of inspired air as it advances into the bronchial tree, as described by measurements in papers in this Thesis(Davies,1975; Horsfield and Davies,1979)

and theoretically by other workers, means that molecular diffusion plays a more and more important role in gas mixing in the lungs as a gas front advances.

It has been suggested from morphometric analysis of the bronchial tree by Cumming, Horsfield and Preston (1971) that when inspiratory bulk flow and molecular diffusion of gas occur in opposite directions the two processes can come into balance. Thus when a breath of oxygen is taken the nitrogen in the residual gas diffuses outwards during the inspiration while convective flow simultaneously washes it back in. If inspiratory flow were constant it is theoretically possible for a stationary front of nitrogen to develop (250ml down the airway in Cumming's model).

The rate of gas mixing in the conductive region of the lung, and hence the establishment of a concentration front under conditions of constant flow, has considerable importance in the diffusive functioning of the respiratory region of the lung. The ability of a stationary concentration front to form during steady flow depends on the anatomy of the airways, in which total cross-sectional area increases with distance from the carina. Mean flow velocity at any point is inversly proportional, and molecular diffusion directly proportional to cross-sectional area, so that with respect to changing distance from the carina one process diminishes while the other increases (Gomez 1965). A balance point is likely to exist. Of course oxygen continues to enter the alveoli during inspiration, moving down due to convective flow and molecular diffusion, so the mean concentration of nitrogen in the alveoli steadily falls. This however has little effect on the position of the front.

The theoretical situation in which there is a constant flow of gas into the lungs on which the calculations which lead to the postulation of a stationary concentration front is a highly artificial one and Fukuchi, Roussos, Macklem and Engel (1976) have shown in both *in situ* and excised dog lungs that cardiogenic pulsations materially affect gas mixing in the airways. The hollow casts described earlier in this Thesis were used to investigate what conditions allowed a stationary concentration front to be established in the bronchial tree and what effect on the overall diffusion coefficient cardiogenic pulsations might have.

In Horsfield, Davies.and Cumming(1980)we describe how nitrogen was blown into the hollow casts in an inspiratory direction and at increasing constant rates while concentration within the cast was measured through holes drilled at 1cm intervals along several pathways from the carina to the periphery. This part of the investigation determined whether stationary fronts could be established at flows found in the respiratory cycle. On these flows was superimposed oscillatory flows which mimicked cardiogenic oscillations which enabled the contribution made by this source to gas mixing in the lung to be estimated.

A flow of $6.7ml.s^{-1}$ satisfactorily established a stationary front of nitrogen 200mm from the carina. The in-flowing nitrogen was subjected to oscillations with a stroke volume of 3.0ml 5.5ml and 8.0ml, each at 1,2,3Hz. Oscillation, and increase in stroke volume or frequency of oscillation moved the front towards the carina.

If gas mixing within the single continuous system of tubes that constitutes the respiratory system were complete at any time during the respiratory cycle composition of gas at the mouth would be the same as that in the alveoli. That is clearly not the case and it is common practice in physiology to divide the airway into two mixing zones, the anatomical or series dead space, broadly corresponding to the conducting airways, and the alveolar region distal to the terminal bronchioles. Our demonstration of the existence of a stationary front during inspiration makes possible a functional definition of the dividing line between the two, series dead space being proximal to the front and the alveolar region distal to it. Gas mixing takes place at the front, diminishing series dead space as it progresses, and reducing alveolar inhomogeneity. Both cardiac oscillations and the use of gases of higher diffusivity result in the front being established higher up the airways, in our experiments we calculate the oscillatory effect had an equivalent effect to increasing the diffusivity of the gases by a factor of five. Engel, Menkes, Wood , Utz and Macklem (1973) showed that in dogs dead space rises by 13% with cessation of heartbeat probably due to the effect demonstrated with the casts. As this effect was restricted to the airways it is likely that a similar or even greater effect is at work in the alveoli .

Section 2.SUMMARY OF STUDIES OF CONTROL OF PATTERN OF BREATHING

Section 2.1 The State of Knowledge in 1970

The work described in this section of the Thesis represents a contribution to the understanding of reflex neural control of breathing, and in particular that related to reflexes carried by myelinated fibres from the lung.

The state of knowledge relating to the effects of the afferent information carried by these fibres at the time this work was started in 1970 is first briefly described.

In attempting to explain the reflex neural control of breathing we have been bedevilled by the inconvenient fact that many complicated interactions and feedbacks always follow simple stimulation of a respiratory reflex.

From almost the turn of the century it has been recognised that suitable electrical stimulation of any somatic and visceral nerve will change breathing (Ranson, 1921) Although most modalities of sensation can have some respiratory action certain afferent pathways can be accorded primacy of control, since they change alveolar ventilation or modify pattern of breathing in response to frequent physiological rather than experimental events. For example the inflation reflex can be considered to be involved in the inflation of the lungs which accompanies each inspiration, and exerts it effect on pattern of breathing in normal intact animals, if least of all in man. Other responses to abnormal or pathological conditions, for example the response to pain or inhaled irritants, are sufficiently common to also warrant consideration as part of the reflex control of breathing. Other reflexes such as those from the arterial baroreceptors have an ancillary or feeble role.

The stimulation of even a primary respiratory reflex is not without confusing results. Such stimulation may alter blood gas tensions, lung volumes, vascular pressures and bronchial smooth muscle tone, provoking many complicated interactions and feedbacks which defeat the search for a clear picture of what each receptor system does.

Despite this limitation it was apparent well before the 1970s that the major reflexes from the respiratory system arise from afferent end-organs in the lungs. A major review of respiratory reflexes in 1964 (Widdicombe,1964) identified eight types of afferent end-organs a) subepithelial receptors, b) smooth-muscle endings, c) encapsulated endings in the respiratory bronchioles and atria, d) unencapsulated endings, e) perichondral receptors, f) pleural endings, and g) receptors in the pulmonary vascular bed; and as an earlier commentator dryly remarked "there is a sufficiency of types of receptor to account for a multiplicity of reflexes" (Elftman,1943).

This "sufficiency of types" served to muddy the waters in the search for a hierarchy of importance of receptors and a clear catalogue of effects of the major types. The main problem lay in the great difficulty in isolating a stimulus to one physiological group of receptors. For example, considering reflexes in general, nerve section indicates the over-all tonic action of the cut nerve. If a reflex disappears after section of a nerve (which does not contain its motor pathway) the interpretation that the nerve contains the afferent fibres of the reflex is only valid if the section has not created new conditions in which the reflex, even with the pathway intact, cannot be elicited. Vagotomy creates a transient example of this in the rabbit where the effects of phenyl diguanide are suppressed for a time after nerve section but reappear (Davies & Jones,1986).

Differential block of nerves, in particular the vagus nerves, appeared to offer the potential to isolate stimuli from specific receptor types, and cooling (Daws, Mott & Widdicombe, 1951) pressure (Leksell, L. 1945) local anaesthetics (Nathan &Sears, 1960) and anodal current (Kuffler & Vaughan-Williams, 1953) had all been used by this time.

Apart from the technical difficulties of measuring temperature, current or drug concentration in a tiny area of tissue all these methods of "differentially" blocking mixed nerves, in particular the vagosympathetic trunk, suffer from their dependence on nerve fibre diameter for their intensity of action. Thus large myelinated fibres are blocked at a higher temperature than small unmyelinated and while there may be a workable separation between myelinated and unmyelinated

that may be used to produce a differential block there is an overlap of fibre diameter in the myelinated category. This means, in the respiratory vagus which we are particularly interested in, a block of the large diameter fibres, from slowly adapting receptors for example, which does not include fibres from rapidly adapting receptors is incomplete, and a block which includes all fibres from pulmonary slowly adapting receptors (PSR) must block some from rapidly adapting receptors (RAR).

The same problems of specificity apply to stimuli. Apart from primary changes in pattern of breathing produced by one type of receptor affecting the activity of others there is the problem of a major stimulus (inflation for example) to one receptor type almost inevitably affecting another.

It is clear why the analysis of respiratory reflexes is difficult, producing results which are often controversial, and why in the early 1970s the understanding of reflex control of breathing was largely based on circumstantial evidence rather than a single conclusive experiment. The aim of investigators at this time was to explain the origin of reflexes produced by experimental inflation and deflation of the lungs, for example, as parts of the normal pattern of breathing along with reflexes such as sneeze cough and sniff provoked by stimulation of the upper and lower airways.

The response to experimentally imposed inflations and deflations of the lung depend to some considerable extent on the nature (rate and volume) of these manoeuvres but for this overview we can consider them under general headings of-

The Inflation Reflexes

Inflation of the lungs or preventing their collapse at the end of inspiration inhibits further inspiration for some time in all species in which this experiment has been tried. The reflex is of different strengths in different species (Bucher,1949; Widdicombe,1961) and is generally agreed to be weakest in man. That vagotomy abolishes this reflex demonstrates its afferent pathway, and the analysis of Adrian (1933)

identified vagal slowly adapting activity which correlated with the inflation reflex. This activity was blocked at temperatures below 8°C (Daws,Mott & Widdicombe,1951) while other respiratory reflexes remained intact. This and graded electrical stimulation (Wyss,1954) identified the fibres carrying this reflex as the Aα and Aβ types. The primacy of the inflation reflex and the receptors that produce it in the *Selbststeuerung* of breathing was rightly acknowledged at this time but largely to the exclusion of other reflexes and receptors except in almost pathological conditions. The conduction velocity or temperature to block fibres associated with all but 2 of 9 reflexes cited in Table1 of Widdicombe's Review (1964)were unknown, as was therefore their receptors of origin. The recognised need to understand receptor activity before a deconstruction of the system could be complete and the consequent priority given to this work is reviewed by Sant'Ambrogio (1982).

The seductively regular and repeatable activity of slowly adapting receptors in the lung and bronchial tree may have focused attention on their contribution to control of breathing, and was incorporated into "off-switch" models of control such as those of Clark and von Euler (1972) almost to the exclusion of other types of activity which were relegated to the realms of pathophysiology.

However, inconveniently well established in the panoply of reflexes recognised at that time was Head's Paradoxical Reflex (Head,H.1889) which was originally described as a stronger and lengthened contraction of the diaphragm of rabbits on inflation of the lungs while their vagus nerves were partially recovered from freezing. The existence of the reflex was verified by such authorities as Larrabee and Knowlton (1946) and Cross and co-workers(1960); but the description of its origin at that time could go no further than- "It is therefore likely that the paradoxical reflex is distinct; its receptors have not been identified" (Widdicombe 1964).

Twenty two years later Coleridge and Coleridge (1986) were able to advance the discussion so far as to state "Head's paradoxical reflex is usually ascribed to stimulation of rapidly adapting receptors", but with the caveat that the reflex

differed from gasps and sighs in being a sustained inspiration and might be initiated by unmyelinated fibres.

At that time the problems of resolving the mechanisms of the paradoxical reflex were compounded by the observation that vigorous inflations of the lung, rather than terminate inspiration, due to the accepted action of slowly adapting stretch receptors, in fact augmented inspiration. It would have been convenient to attribute this augmentation to rapidly adapting receptors (at that time usually known as "irritant" or "deflation receptors") which were clearly stimulated by the inflation (Widdicombe, 1954) however the role of rapidly adapting receptors at that time was clearly relegated to a response to powerful exogenous irritants and the cough reflex (Widdicombe, 1954a, 1954b) while (at the time recent) data concerning activity from C-fibre receptors, identifiable with present J-type receptors but then called deflation receptors (Paintal,1957),a classification which persisted for over ten years (Coleridge, Coleridge, Luck and Norman,1968) did not help to clarify the role of RARs in that reflex.

The Deflation Reflexes

Reduction in lung volume by any method so far used (negative pressure breathing, pneumothorax, chest compression) causes reflex tachepnoea in all species in which the experiment has been tried. This reflex arises mainly from the lungs although there is a component from the chest wall (Bland, Lazerou Dyck and Cherniak, 1967) and from sympathetic afferents if the deflation is almost pathologically extreme (Torrance and Whitteridge, 1948).

The reflex is almost exclusively vagal and consists of both a shortening of t_I and t_E. The concomitant reduction of V_T is probably due to mechanical restriction because we have demonstrated the effects on peak phrenic activity are clearly excitatory (Davies *et al* 1978).

Blocking with cold (Hammouda andWilson, 1939. Koller and Ferrer, 1970) and anodal polarisation (Guz and Trenchard, 1971) suggest that non-myelinated fibres do not contribute to the deflation reflex. Direct recording from the vagus nerve confirms this conclusion and demonstrates that, unlike the effects of inflation,

deflation produces a sustained, if phasic, receptor stimulation (Sellick and Widdicombe, 1971).

At this time, the mid 1970s, evidence suggested that there were two vagal components to the excitatory effects of lung deflation, one which increased frequency and one which increased respiratory drive, as expressed by peak phrenic activity in a breath. There was at that time however no reliable way of separating these two components.

Head's Paradoxical Reflex

Several types of inflation manoeuvre in a variety of species produce inspiratory efforts which at the time of their first observation, and for several years after, were considered puzzling or even "Paradoxical". The earliest, observed by Head (1889) consists of a sustained contraction of the diaphragm in response to moderate inflation of the lungs in rabbits in which the vagi are being re-warmed.

This has been tenuously related to the gasp reflex of cats (Larrabee and Knowlton, 1946) and to the gasps elicited from babies by Cross *et al.*(1960).

That RARs are implicated in all of these reflexes is now clear. The extent to which unmyelinated fibres are involved is still open to debate although the paradoxical results of Head are most likely of any to show their effect (Guz and Trenchard, 1971).

As in the case of the deflation reflex, elucidation of the origin of the Paradoxical Reflex was hindered by the lack of an adequate separation of the afferent input that initiates it.

Upper Airway Reflexes

These include nasal and pharangeal reflexes and reflexes from the larynx. One of the daunting features of the study of naturally occurring reflexes from the upper respiratory tract is that it is probably very rare for a stimulus to activate a reflex from only one site. Patterns of reflex response are manifold and apart from the obvious protective and defensive interruptions of the pattern of regular breathing (Widdicombe,1986) include cardiovascular (Daly,1986) and skeletal muscle and spinal reflex effects (Coleridge and Coleridge, 1986).

As long ago as 1922 Jackson observed that while a small irritation of the larynx or trachea causes vigorous coughing, the lungs can sustain considerable invasion by foreign material without reflex excitation. The nature of the response to mechanical stimulation differs with the point of application also. In the uppermost airways provoking cough or sneeze which converts to rapid shallow breathing as the stimulus is applied deeper in the lungs. These protective reflexes are superimposed on the normal pattern of breathing, and take over that pattern, for example in the long drawn out inspiration that precedes a cough. We have demonstrated that these reflexes are perturbed when one of the neural control mechanisms of normal breathing is removed (Sant'Ambrogio,F.,Sant'Ambrogio,G. and Davies,A. 1984) The discovery of a highly specific method of blocking one of the vagal inputs to the reflex control of breathing provided us with a most useful tool.

Section2.2 Blocking PSRs

I have discussed in the previous chapter how a major problem in investigating respiratory reflexes is one of isolating a stimulus to one physiological group of receptors, or alternatively removing the activity of one group of receptors to identify that group's role.

If it could be done in a highly specific manner the latter technique would have the advantage of identifying the contribution made by the receptor group in question to the response to physiological stimuli such as volume and pressure changes in the lung without provoking secondary changes in other systems, changes which produce problems we have already addressed.

While investigating the effects of inhalation of gaseous sulphur dioxide (SO_2) on anaesthetised rabbits with the intention of using it to produce a model of bronchitis we were surprised to find that it highly specifically blocked the activity of pulmonary stretch receptors (PSR). The stimulating action of (SO_2) on rapidly adapting receptors is well known (Widdicombe , 1954) but in the concentrations used in our experiments (200 parts per million) this stimulation is transitory.

Many stretch receptors are blocked within 30 seconds of the animal beginning to breathe the gas, and the 10 minute exposure we have used in our experiments is rather arbitrary.

The mechanism by which this block occurs is not yet known but histology suggests it is not due to frank damage of the airways. We have speculated that since ammonia, an alkaline gas, stimulates stretch receptor activity, CO_2 a weakly acid gas inhibits their activity (Bartlett and Sant'Ambrogio, 1976) and SO_2 blocks them it may be a pH effect. Alternatively the fact that sulphate ions (SO_4^{2-}) are relatively non-diffusable compared to carbon dioxide may have some bearing on the matter.

Another interesting but unpublished observation is that the effect is species specific, being much more potent in rabbits than in dogs and cats.

Our initial full report of this phenomenon (Davies,. 1996) described how both the receptor activity and the Hering-Breuer reflex we used as a physiological test for functional block returned after 30 minutes to an hour and the block could be re-applied almost as often as required. Receptors became silent in different ways, some were initially excited increasing their peak frequency of discharge with each inspiration before finally silencing. Others stuttered and became silent while others abruptly silenced. We speculate these differences may have been due to their different locations and therefore exposures.

What ever the cause of stretch receptor block the effects on pattern of breathing and lung reflexes was well defined. Duration of inspiration (t_I) and tidal volume (V_T) increased and these variables increased further on vagotomy. This phenomenon has been observed in other forms of vagal block (Karczewski and Widdicombe, 1969)

This observation suggests there is a vagally-dependent control of t_I and V_T by a pathway other than that of the inflation reflex. It is unlikely that this effect is due to residual PSR activity because of the changes in expiratory duration (t_E) in the sequence intact-blocked-vagotomised which will be discussed shortly and because there was no inflation reflex in response to large inflations of the lungs, a more potent stimulus than tidal volume excursions.

Stretch receptor block in this series of experiments caused a decrease in t_E. Subsequent vagotomy caused an increase in t_E to a value greater than the control. Substantial evidence (Clark and Euler,1972; Knox,1973) suggests that PSR may be responsible in part at least for the control of t_E . The increase in t_E seen with vagotomy after PSR block suggests the activity of RAR or J-receptors was shortening t_E . This explains one of the two paradoxes current at that time that if PSR were the only important influence on normal quiet breathing , which was suggested by many, vagotomy would be expected to shorten t_E when in fact it lengthened it. The other paradox, relating to the changes in t_I and also based on the supposed exclusivity of PSR control, is dealt with later in this section.

In PSR blocked rabbits increase in breathing frequency brought about by carbon dioxide consisted of reduction in t_I and t_E supporting the suggestion of Widdicombe and Winning (1974) that intact vagal circuits are not essential for this effect.

Stretch receptor block modified but did not abolish the respiratory response to intravenous phenyl diguanide (PDG). The reduction in t_I produced under control conditions was converted to a small increase. It may be that the block of PSR interfered with the effect of the increase in FRC that injected PDG produces. However the received wisdom of the time (1977) that the action of PDG is exclusively peripheral has been thrown into some doubt by later experiments on the effect of vagotomy on this phenomenon (See Section 2.10).

Intravenous injections of histamine produced almost identical ventilatory responses before and after PSR block, which suggests that although histamine sensitises PSR (Widdicombe, 1961) its main action on pattern of breathing is via RAR or unmyelinated fibres and is to shorten t_E and to a lesser extent t_I.

It was the effects of induced pneumothorax in these experiments that provoked the most interest as they appeared paradoxical. During pneumothorax stretch receptor activity is decreased (Knowlton and Larrabee,1946) J-receptors are only slightly stimulated and then only by extreme degrees of collapse (Paintal,1973). During induction of a pneumothorax in an intact animal t_I would frequently be prolonged into a single "augmented breath" consisting of a prolonged t_I followed

by rapid shallow breathing. During removal of the pneumothorax there would be no augmented breath and no rapid shallow breathing. The effects of inducing or removing a pneumothorax on RAR are qualitatively the same- stimulation, the effects on intrathoracic PSR are diametrically opposite- inhibition on induction, stimulation on removal. These differences in PSR activity were used by many people in an attempt to explain the selective production of augmented breaths on induction of pneumothorax and the difference in pattern between these augmented breaths and the rapid shallow breathing that followed them during deflation of the lungs. Interestingly, but inconveniently for this explanation, this general pattern of augmented breaths and rapid shallow breathing survived block of PSR with SO_2. Ruminations on our part as to whether the pattern of RAR discharge was different at the induction of a pneumothorax from that at its removal did not provide a satisfactory explanation and we were left with the observations-

1) Duration of t_I on block of PSR reached a value intermediate between normal and the vagotomised value. (something was holding down t_I when PSR were blocked).

2) Stimulation of RAR seemed to be able to produce an augmented breath (a deep slow breath, a sigh) and also produce rapid shallow breathing in which the acceleration was mainly a shortening of t_E. (RAR produced diametrically opposite patterns to apparently the same stimuli).

What was holding down the duration of inspiration during PSR block when these classical terminators of inspiration were removed and the effect of the only active receptors (RAR) seemed to be to extend t_I into an augmented breath ? And how could these receptors produce what was essentially diametrically opposite patterns of breathing, deep and slow or rapid and shallow?

Section 2.3 Stimulating Rapidly Adapting Receptors.

The blocking of PSR with SO_2 leaves RAR and receptors associated with unmyelinated fibres unaffected and exposes their effect on pattern of breathing under the conditions prevailing at the time, in our experiments usually quiet anaesthetised breathing.

To exaggerate and thereby hopefully make clearer the effects of these receptors on breathing we devise methods of specifically stimulating them. If these stimuli are themselves exaggerations of normal physiological stimuli we may be justified in associating the effect we have produced with normal pattern of breathing. This is why such "brutal" unnatural stimuli as injected histamine and diguanides, and pneumothorax are of dubious use in elucidating the fine detail of reflexes controlling breathing.

In this series of experiments we therefore used brief pulses of inflation or deflation of the lungs, an exaggeration of the process of breathing, to stimulate RAR in intact and PSR blocked rabbits. We have observed in abstract (Davies & Roumy,1997) and Pack and Delaney (1983) later confirmed that the specific stimulus for RAR is rate of change of volume of the lung (flow). Although it had been known for some time (Mills,Sellick & Widdicombe, 1969) that they are strongly excited by both inflations and deflations of the lungs.

The beauty of brief (100ms) pulses of inflation or deflation as a stimulus is that inflation and deflation produce diametrically opposite effects on intrapulmonary PSR, one stimulating the other reducing activity. Extra-pulmonary PSR behave in a different way as demonstrated by Bartlett, Jefferys, Sant'Ambrogio and Wise (1976) and were excluded from our experiments by the method of preparation. Therefore any effect produced by both pulses of inflation and deflation could not be attributed to PSR because of the diametrically opposed effects of these two stimuli on their activity.

As far as J receptors and C-fibre endings are concerned it is known that inflations of up to four times tidal volume fail to excite J-receptors (Paintal,1969), and C-fibre endings show similar volume insensitivity (Coleridge & Coleridge, 1977).

In 1981 we therefore combined block of PSR in anaesthetised rabbits with pulses of inflation and deflation of their lungs in an attempt to resolve the apparent

paradox of RAR producing slow deep augmented breaths and rapid shallow breathing (Davies & Roumy,1982).

Augmented breaths had already been ascribed, at least in part, to the then so called "irritant receptors" (Reynolds,1962).

However histamine, in the form of aerosol or intravenously injected, accelerated breathing by reductions in t_I and t_E . (Widdicombe & Winning, 1976) and histamine stimulated RAR. We therefore combined our highly specific block of PSR with our method of stimulating RAR to try to determine their role in augmented breaths and accelerated breathing. One of the advantages of our pulses of inflation and deflation as a stimulus was they were sufficiently brief (100ms) to be placed at many different points in the respiratory cycle and so determine the sensitivity of the respiratory pattern generator to RAR input at different times in a single breath.

Firstly dealing with stimulation in inspiration- this produced augmented breaths which were identical in every respect with those that occurred spontaneously, consisting of a biphasic inspiration of duration 1.75 control t_I. The tidal volume was larger than normal but would have been distorted by the injection or removal of volume by the pulse. Augmented breaths could be provoked whether PSR were blocked or intact and whether the pulses that produced them were of positive or negative pressure. The triggering of augmented breaths was an all-or-none affair. Pulses in inspiration either provoked an augmented breath or produced no significant change in duration of inspiration. This can be explained by the pulse being too brief to exert much influence on the total integrated stretch receptor activity when PSR were intact. This seems to provide incontrovertible evidence for RAR as the origin of augmented breaths.

Experiments with anaesthetised paralysed rabbits also revealed information about the structure of augmented breaths. In these animals, which of course have to be artificially ventilated, phrenic activity is relegated to the deflation phase of the lungs by PSR activity (if present) during the inflation phase. In PSR blocked rabbits phrenic activity "runs free" below a certain threshold of pump stroke-volume (see Section2.6 of this dissertation) . In these paralysed rabbits a pulse

during phrenic silence produced a short latency burst of phrenic activity of duration $0.75t_I$. This would explain how pulses in phrenic activity in the paralysed animals produced variable increases in t_I up to a maximum of 1.75 control t_I , the phrenic activity in these cases was a normal discharge with the short ($0.75t_I$) burst superimposed; the total duration of t_I being determined by the degree of "overlap" of its two parts. This also explains the highly consistent duration of augmented breath t_I in the un-paralysed rabbits (1.75 control t_I) as a control inspiration plus the phrenic burst. This description explains both the all-or-none and the biphasic nature of the augmented breath.

Perhaps the most important feature of the results obtained in these experiments was the demonstration of a refractory period of about 2minutes after an augmented breath, during which it was impossible to provoke a further one. A similar phenomenon has been reported by Reynolds (1962) but was restricted to the observation that augmented breaths were more difficult to provoke sequentially. The importance of the data produced in this series of experiments is that it demonstrates the refractoryness is restricted to the augmenting of inspiration, the shortening of expiration, which makes up the largest part of the acceleration of breathing brought about by RAR stimulation, is not refractory and can be produced in any number of consecutive breaths, as we will see in the next section.

The brevity of the RAR stimulating pulses enabled us to insert them into any different part of the respiratory cycle i.e. we could stimulate the beginning middle or end of expiration. The response when the pulses were inserted into the expiratory phase was less stereotype than when inserted in inspiration, and depended on whether PSR were intact or blocked, whether the pulse was of negative or positive pressure and the position of the pulse in expiration.

In the PSR intact rabbit pulses of inflation in the second half of expiration produced lengthening of t_E , in the first half they produced shortening as did inflations at any time in expiration when PSR were blocked or when the pulses were of negative pressure.

Previous workers had demonstrated that deflation and inflation of the lungs shortened and lengthened t_E respectively(Knox,1973), these effects were attributed to PSR. Our experiments, by blocking PSR and stimulating RAR showed their activity terminated t_E with a constant latency, the apparent graded effect we produced is due to the positioning of the pulse rather than different potencies. These experiments demonstrate that the control of t_E is a balance between the extending effects of PSR and the terminating effects of RAR. From these experimental results we can summarise the effects of RAR on breathing as extending inspiration into an augmented breath , an effect which is refractory over a period of minutes, and terminating expiration with a constant latency, an effect which is not refractory.

This description of the control system explains how RAR can produce two different patterns- the slow deep augmented breath with its extended t_I which is not seen again for some time and rapid shallow breathing with its shortened t_E which persists for the whole while RAR are stimulated.

This description does not explain the pattern of breathing seen during the steps from intact through PSR block to vagotomy, when inspiration lengthens with PSR block but does not reach its vagotomy value. To put the question another way **"what is holding down t_I when PSR are blocked when all we have been able to prove RARs do is <u>extend</u> t_I into an augmented breath".**

To answer this question we carried a very simple series of experiments in which we measured t_I , t_E and V_T of several breaths in anaesthetised rabbits whose stretch receptors were first intact, then blocked and then both vagi cut while the animals breathed CO_2 to accelerate their breathing . The purpose of the accelerated breathing was to provide a number of examples of individual breaths under the three conditions of vagal feedback. We were by now convinced that the reflex changes in pattern of breathing provoked by sustained inflations and deflations of the lungs, used by us and other workers as tests of reflex activity, contained a considerable component of RAR activity and we included in this series of experiments sustained inflations and deflations of the lungs to asses the relative

contributions of RAR and PSR to these reflexes. Our results are reported in Davies and Roumy (1986).

I can summarise our reasoning thus- If pulmonary stretch receptors were the major vagal determinant of pattern of breathing under the circumstances of our tests, a block of their activity should have produced a breathing pattern almost identical to that seen in vagotomised animals, it did not.

In our experiments we plotted the V_T, t_I, t_E relationships for individual breaths Figs 1&2 (Davies and Roumy,1986) thus in the Figures. Each V_T will have its t_I (in Fig 1) and t_E (in Fig 2) associated with it.

Shift to the right by the V_T-t_I curve produced by PSR block provides further evidence of the importance of these receptors in control of inspiratory duration. For a given tidal volume PSR block caused an increase in t_I which is in agreement with the concept of an "off-switch" mechanism. But again, in this series of experiments we see that t_I only reached a value intermediate between intact and vagotomised.

The V_T-t_E relationship contrasted with the V_T-t_I relationship outlined above. Unlike with t_I during PSR block points describing V_T-t_E during block lay on a continuation of the curve describing this relationship before the block (Fig 2. Davies and Roumy,1986) unlike the V_T-t_I relationship which was displaced by the block.

That the V_T-t_E curve was altered by vagotomy after PSR block suggests that receptors other than PSR were involved in its control and I have already described how we are of the opinion that these are RAR.

Sustained lung deflation produces a large persistent increase in breathing frequency in rabbits due to a decrease in t_E. At this time (1986) this was being attributed to a decrease in PSR activity (Knox,1973) and even to the postulated accelerating effects of low frequency PSR activity (Paintal,1973 for Review). Any transient increases in t_I immediately on deflation was due to augmented breaths whose genesis we have dealt with. Non augmented t_I was not different from control. Sustained deflating pressures of 0.5K Pa produced a shortening of t_E which was 50% due to the activity of RAR. Similarly inflation of the lungs with a

pressure of 0.5 K Pa , which normally produces a lengthening of t_E (the Hering-Breuer reflex) produces a shortening of t_E to 75% control when PSR are blocked, probably due to stimulation of RAR. We still could not however subscribe to the idea of a direct t_I shortening effect of RAR activity, or that of any unmyelinated fibre activity producing such an effect.

In a previous paper (Davies and Roumy,1982) and in the papers cited in the next section of this Thesis we reported that when t_E was shortened by RAR activity there was a shortening of the following t_I . This took place whether PSR were blocked or intact. Karczewski *et al* (1976) reported electrical stimulation of the vagus nerve of spontaneously breathing anaesthetised rabbits produced changes in pattern of breathing in which reduction in t_E preceded reduction in t_I in the first breath of the change. It had been postulated for some time that the conditions at the end of inspiration, and even during inspiration, would affect the following expiration. We suggest that there is a central "linking" which works between both phases of the respiratory cycle i.e. each t_I affects the next t_E and each t_E affects the next t_I.

By postulating this, at least partial, influence of t_E on the next t_I we can explain the control of t_I at the three stages of our experiments without evoking the concept of a t_I limiting influence of RAR. When PSR were blocked there was an increase in t_I which should have assumed its vagotomy value if they were its only modulator. However the removal of PSR and the RAR activity shorten t_E . The increase in t_I predicted on the removal of PSR was limited to a value fixed by the central t_I-t_E relationship and the point on that relationship fixed by the value of t_E.

According to our theory-

1. The existence of a central t_I-t_E relationship means these two variables of individual breaths in PSR blocked and vagotomised animals will lie on a common curve.
2. In intact rabbits PSR activity will displace the variables for individual breaths away from the curve.

Both these predictions were fulfilled by the results in summarised in Fig-Fig 5 Davies and Roumy(1986).

We concluded that duration of inspiration and expiration in our experiments was basically determined by a central pattern generator which links these two variables, perhaps in a way shown in Fig 6 although the exact shape position and influence of other factors such as temperature and blood gases is not essential to our argument. Block of PSR allows the point describing a single breath to move onto the line by an increase in t_I and a decrease in t_E. The major modulation of t_I is now the t_I-t_E relationship and the exact position on the central relationship is determined by removal of PSR and activation of RAR restricting t_E . This restricted t_E in turn holds down t_I to a value less than that in vagotomy.

Our theory reconciles the two apparently contradictory effects of RAR-Production of augmented breaths with prolonged t_I and production of rapid shallow breathing with reduced t_I.

During intense RAR stimulation, such as during pneumothorax, there is a single lengthening of t_I to produce an augmented breath. There is then a prolonged period during which the t_I lengthening mechanism is refractory. During this period t_I is shortened by the central t_I-t_E linking and a reduced t_E.

An essential requirement of this thesis is that there be a linking between t_I and t_E. The experiments cited in this section of the Thesis provided evidence for this and we have other evidence for the existence of such a link from experiments described in the next section.

Section 2.4. Continuous & Discontinuous Breathing

Although drugs have provided valuable tools for studying reflexes from the lungs for many years (Dawes and Comroe, 1954) only relatively recently the confounding effects of ignoring the influence of different routes and rates of administration have been fully recognised. Bolus intravenous administration or powerful aerosols of histamine rapidly accelerate breathing in rabbits at such a rate that the peak frequency of breathing is reached in a few breaths. This unseemly haste has obscured some interesting facts about the different patterns of breathing that can develop as a result of this form of stimulation.

The experiments reported in this section of this Thesis were carried out at about the same time as those in the previous section (Section 2.3) and the results combine to form the basis of our suggestion of a central linking of t_I to t_E which is outlined in that section.

Histamine, blood born or inhaled, stimulates all three systems of lung receptors, stretch receptors (Widdicombe, 1961) Rapidly adapting receptors (Mills,Sellick and Widdicombe, 1969), and C-fibre receptors (Coleridge and Coleridge1977), although the contribution of C-fibre receptors is rather small since block of myelinated fibres (Karczewski and Widdicombe, 1969) largely blocks the effect (But see Section 2.10 of this Thesis).

In all our experiments we found that acceleration of breathing due to histamine was mainly the result of shortening of t_E , but with a significant component due to t_I. The quantitative difference between our results and those of Miserocchi *et al.* (1978), who found a larger reduction of t_I than we did with histamine aerosol, is probably due to the different control conditions involved. Our rabbits were more lightly anaesthetised, with t_I of 0.48s before and 0.6s during stretch receptor block, while Miserocchi's rabbits breathed with t_I between 0.7 and 0.8s with stretch receptors intact (stretch receptors were not blocked in their experiments).

In our experiments the majority of overall acceleration of breathing due to histamine was due to a reduction in t_E both before and after stretch receptor block, this is because t_E is almost always the longer phase of breathing, and therefore able to make a greater contribution to shortening total breath time.

Reduction of t_I occurred before and during stretch receptor block placing its origin, directly or indirectly, firmly with rapidly adapting receptors.

Acceleration of breathing fell into two quite distinct types, with some common features. The first change in breathing seen in either type was <u>always</u> a shortening of t_E. After a delay of several breaths (mean =12), t_I started to change. This change in t_I could be smooth and gradual or there could be no change in t_I until abruptly initiated by an augmented breath . In such a pattern t_I was not changed

from the control value until the augmented breath. We called these patterns "continuous " and "discontinuous acceleration". With stretch receptors intact discontinuous acceleration occurred twice as often as continuous, with stretch receptors blocked the two types occurred with equal frequency. Interestingly if we "injected" an augmented breath by the pulse method described in the previous section we could ensure that the type of response to histamine would be discontinuous.

Although our previous experiments with pressure pulses (Section 2.3) have demonstrated that rapidly adapting receptor activity shortens t_E its only effect on t_I is to provoke an augmented breath- a large increase in t_I . This is an all-or-nothing effect; levels of rapidly adapting receptor activity which do not provoke an augmented breath do not alter t_I . If rapidly adapting receptor activity extends t_I , as in an augmented breath, what shortens t_I during histamine administration, particularly when stretch receptors are blocked ? The contribution of C-fibre receptors is rather small since the histamine effect is abolished by differential block of all myelinated vagal fibres. We suggest that there is a linking of the central t_I determining mechanisms to those which control the already shortened t_E and this brings about the shortening of t_I.

It is clear from the way changes in t_E precede those of t_I in continuous acceleration, and from the existence of discontinuous acceleration, that t_I and t_E are independently controlled over at least part of their range. That they can be linked, with t_E influencing t_I is suggested by the major part of continuous acceleration . This linking may be the direct effect of factors shortening t_E on t_I or the response of the independent neural systems controlling these variables to a common input. If the latter is the case sensitivity of the mechanism controlling t_I to the effect of t_E must change dramatically after the augmented breath where t_I changes from control to near minimal value in a matter of one or two breaths. We asked ourselves " What brings about this change or linking?" It can not be an effect of stretch receptors because it occurs whether they are intact or blocked. Could it be mediated by rapidly adapting receptors ? The only direct influence of rapidly adapting receptors on t_I we have been able to demonstrate is the

production of an augmented breath. (Davies and Roumy,1977) which consists of a lengthened t_I .

Our thesis therefore is that the neural mechanisms that allow t_E to influence t_I may not be functional until rapidly adapting activity reaches a level which provokes an augmented breath (this is discontinuous acceleration) then the already shortened t_E begins to limit t_I . In continuous acceleration the linking mechanism is functional from the start, perhaps already initiated by the existing level of rapidly adapting receptor activity.

It is interesting that provoking an augmented breath with a pulse of pressure produces an exact replica of spontaneously occurring discontinuous accelerated breathing. It is not clear whether it is the level of rapidly adapting receptor activity, such as is sufficient to provoke an augmented breath, or the mechanisms involved in the augmented breath itself that promote the linking.

Probably because they were interested in the maximum effect produced rather than the dynamic situation leading up to the maximum effect other workers have not commented on the types of tachypnoea produced by histamine. However the effects are clearly there in other peoples work. For example the study of Winning and Widdicombe (1976) clearly shows in their Fig.5 discontinuous acceleration, although it is not commented on.

This series of experiments showed that in rabbits, with or without pulmonary stretch receptors intact, t_E can change independently of t_I over part of its range, and that breathing can accelerate in two distinctly different ways. This "indirect" influence of rapidly adapting receptors on t_I explains the intermediate state between intact and vagotomised condition described in the previous section.

Section 2.5 Postural Changes

The effect of gravity on the physiological systems of the body will probably be familiar to most people above a certain age who have risen rapidly from a recumbent position and experienced the sensation of orthostatic hypotension.

This effect of gravity and the fact that the cavities of the thorax and abdomen are only separated by the very flexible diaphragm is brought home by the effect of gravity on the weighty abdominal contents. The effects of this on respiration were reported many years ago (Moruzzi,1945).

It has also been noted that, in the rabbit at least, these reflexes are exaggerated by anaesthesia (Sant'Ambrogio and Widdicombe, 1965). This communication is a two-way affair, with increases in lung volume provoking powerful reflexes which activate the abdominal muscles (Bishop,1964)

Inflation of the lungs and tilting into the head-up position produces an increase in Functional Residual Capacity (FRC), cessation of inspiratory activity and activation of the internal oblique muscles of the abdomen. This is followed by breathing with decreased tidal volume, which can be explained by the inspiratory muscles working on a different part of their tension-length relationship (Agostoni,1964;Marshall,1962;Sant'Ambrogio and Sabiene,1970)

The increase in Functional Residual Capacity, arrest of inspiration and increase in activity of the abdominal muscles provoked by inflation of the lungs and head-up tilting is a vagal reflex ,as demonstrated by vagotomy. That it involves pulmonary stretch receptors (PSR) might be suspected from the nature of the stimuli and the sustained nature of the response, a conclusion supported by the observation of Mortola and Sant'Ambrogio (1973) that partial block of the vagus by direct current suppresses the response. We decided to use the highly specific block of pulmonary stretch receptors produced by SO_2 to identify the contribution made by these receptors.

We found that positive pressure breathing and head-up tilting that produced similar increases in FRC produced much greater activation of the abdominal muscles in the case of tilting than inflation. Although we have not unequivocally identified the origin of this potentiation Bishop (1964) demonstrated facilitatory influences of passive stretching on abdominal muscle contraction, which implicate these muscles rather than the viscera in this effect.

Whatever the origin of this input it was incapable of activating the abdominal muscles by itself, vagotomy or PSR block abolished the effect of tilting.

The absence of this abdominal reflex explains the greater shift in FRC during PSR block, there are no changes in compliance (the most likely other cause) produced by vagotomy that could produce this shift (Karczewski and Widdicombe, 1969). The reflex, whose origin we have demonstrated, has clear homeostatic advantages in minimising potential changes in FRC which might be produced by changes in posture.

Section2.6 The Onset of Inspiration

From the beginning of the studies of reflex neural control of breathing that make up this Thesis we formed the clear impression that the two major pulmonary receptor systems associated with myelinated fibres in the vagus nerve made up a "balanced" control system; with slowly adapting receptors (PSRs) inhibiting inspiration and therefore, by default, extending expiration. Rapidly adapting receptors (RARs) we found terminated expiration and provoked augmented breaths (Davies and Roumy, 1977 &1978).

Pulmonary rapidly adapting receptors had been generally neglected by investigators of reflex control of breathing in favour of PSRs whose seductively regular and abundant discharge perhaps suggested a more powerful influence than the sparse and irregular discharge of RARs. The lineage of RARs is however almost as ancient as that of PSRs with the epithelial endings of the rapidly adapting nerves being first described by Larsell (1921); and the activity of these nerves being recorded some eight years later (Keller and Loeser,1929).

Part of the problem of establishing a role for RARs in normal quiet breathing seemed to be a conviction among investigators that their role was restricted to pathology. (Mills, Sellick and Widdicombe, 1969) or at the most to producing gasps or sighs (Knowlton and Larrabee, 1946; Reynolds, 1962; Sellick and Widdicombe, 1970).

The apparently inspiration provoking nature of RARs lead us to design a series of experiments to see if RARs exerted any influence on the onset of inspiration as well as on its character.

The nature of these experiments was essentially simple, we compared the "pattern of breathing" of anaesthetised paralysed rabbits ventilated by a cyclic pump with a pattern that closely matched their spontaneous breathing for frequency and volume, both before and after blocking PSRs with SO_2 (Davies *et al.*,1978). Because the rabbits were paralysed we used the discharge of a root of the phrenic nerve to describe "inspiration" and "expiration", and in particular the start of "inspiration"- the beginning of phrenic discharge, in the ventilatory cycle

In intact rabbits the onset of phrenic "inspiration" was inevitably in the deflation phase of the pump. When PSRs were blocked "inspiration" was either free running, having no fixed relationship to the pump or locked to a particular phase, usually inflation.

Free running discharge could be locked to the pump pattern by a modest (never more than 30%) increase in pump tidal volume; and locked pattern could be disconnected by reducing tidal volume.

After bilateral vagotomy the pattern was always free running.

The well established role of PSRs in inhibiting inspiration is once again demonstrated by the relegation of "inspiration" to the deflation phase in the intact rabbits in our experiments. This was first demonstrated by Tang, Maire and Amassian (1957).

We used SO_2 to abolish the activity of PSRs and the absence of any inspiratory inhibition during pump inflation of the lungs is equivalent to the absence of the Hering-Breuer reflex we use to test the completeness of the receptor block.

The most usual pattern of phrenic discharge seen with receptor block when tidal volume was set at approximately that of spontaneous breathing was free running, the sort seen in the vagotomised condition. This demonstrated there was no significant linking input from other vagal or extravagal sources. However the locking effect of increasing tidal volume could not be achieved when the vagi were cut, demonstrating that this was not an extravagal input.

Of the remaining vagal receptors in the PSR blocked state J-receptors are unlikely to be responsible for the synchronisation because they have been shown to have no tonic reflex effect on breathing in rabbits with healthy lungs (Guz and

Trenchard,1971);their most likely stimulus is lung congestion (Paintal, 1973); they need considerable degrees of lung inflation to be stimulated (Sellick and Widdicombe,1970) and their discharge is normally sparse and irregular (Coleridge and Coleridge, 1977).

In this series of experiments, as in our other previous experiments, RAR activity seems to initiate phrenic activity without any detectable (by us) influence on its duration. Rapidly adapting receptor activity can provoke an augmented breath- which is the addition of an extremely consistent burst of phrenic activity to a normal phrenic discharge (Davies and Roumy,1978) or terminate expiration (Davies, 1978). What it does not seem to do is to alter the rate of build up of activity which has been already determined by some other mechanisms. Winning and Widdicombe (1976) came to the same conclusion from their work with cats. It has been suggested that the onset of inspiration depends on a waning inhibition of PSR origin (Clark and Euler,1972), the excitatory element coming from chemical drive. This explains why inspiration starts but does not explain why, in this series of experiments at least, inspiration could be made to start at the same point in a ventilatory cycle which was not very different from eupnic breathing. Under these conditions RARs are active at functional residual capacity.

It has been observed that the specific stimulus for RARs is rate of change of volume of the lungs (Davies,1978; Pack and Delaney,1983) and the initiating mechanism we describe here would be particularly effective in patterns of breathing without an expiratory pause where RAR are stimulated by rapid deflation of the lungs to end expiratory level.

The results of this series of experiments reinforced the concept of RARs as an inspiratory initiating expiratory shortening mechanism.

Section 2.7 Cough

In a subsequent section (2.9) of this thesis I will describe experiments with conscious dogs in which their vagus nerves were blocked by cooling .
These dogs were part of a series of experiments investigating pattern of breathing and to measure respiratory flow a cuffed endotracheal tube was inserted through a permanent tracheostomy. The tube was removed immediately at the end of the experiment. I noticed that these dogs usually coughed on removal of the tube if PSRs were not blocked. If PSRs were blocked the dogs looked slightly uncomfortable for a moment, increased their breathing frequency, but rarely coughed. In experiments in which we used SO_2 to produce a block of PSRs in rabbits we also noticed they did not cough in response to tracheal stimulation only if PSRs were blocked.
In the experiments described in this section of the Thesis we investigated if this effect of blocking cough was in fact related to blocking PSR activity by using two species, one in which we can block PSRs with SO_2 (rabbits) and one which seems immune to this effect (dogs). We provoked cough from two sites, the larynx and the trachea as cough is usually initiated by stimulation of mucosal endings found from the larynx down to the tracheal bifurcation, These endings can be identified by their rapidly adapting discharge to mechanical stimulation (Widdicombe, 1977).
Pulmonary stretch receptor block has previously been implicated in anti-tussive effects by other workers. Bucher (1956) and earlier Bucher and Jacot (1951). Bucher suggested that the effects of the cough remedy *Tessalon* were the result of an effect on nerve endings by this drug carried in the blood-stream. The work of Sant'Ambrogio and Sant'Ambrogio (1982) on the circulatory accessibility of receptors renders this explanation unlikely.
The muscular act of cough involves several groups of respiratory muscles, inspiratory expiratory and laryngeal, in a highly co-ordinated pattern in which a deep inspiration is followed by a forceful expiratory effort, usually initially against

a closed larynx which opens suddenly to release a blast of air at velocities which can approach the speed of sound (Korpas and Tomori,1979).

A major criticism of our use of SO_2 to block PSR in this investigation of cough might have been that we were in some way damaging the RARs which are agreed to be the origin of the reflex. To pre-empt this criticism we provoked cough from both tracheal and laryngeal sites (SO_2 was administered via a tracheal cannula) and directly recorded from RARs in the trachea before and during block of PSRs in rabbits.

In summary our dogs coughed in response to stimulation of either site before and after administration of SO_2 (which does not block PSRs in this species, and in fact slightly potentiated the Hering-Breuer reflex). In the rabbits PSR block effectively abolished cough from the trachea and significantly reduced (95% to 70%) the chance of provoking a cough from the larynx.

It seems that in rabbits the cough reflex is unique among the respiratory reflexes, other than the Hering-Breuer reflex, in being abolished by block of PSRs, all other reflexes we have tested (Davies, Dixon *et al.*,1978 & Davies, Sant'Ambrogio and Sant'Ambrogio, 1980) have survived the block.

It should be remembered that in dogs vagal cold block, which blocks PSR activity blocked cough while SO_2 which in this species does not block PSRs does not .

Although this series of experiments achieved their objective in clearly demonstrating the involvement of PSRs in the genesis of cough they raised as many questions as they answered, and created new ones.

Why do we not obtain a cough when direct recording demonstrates the viability of RARs? Are PSRs a primary agent in the act of coughing? At what point in the reflex is the contribution of PSRs essential?

The other peripheral afferent pathway that might be involved in this phenomenon is that of C-fibres. C-fibre receptors are stimulated by probing of the mucosa . However C-fibres are stimulated not blocked by SO_2 (Coleridge, Poore *et al.*1982) and cold block of the vagi to a degree that leaves C-fibre conduction intact blocks cough (Widdicombe,1977). A central action of SO_2 is highly unlikely

as the dogs in these experiments were exposed to a higher concentration and therefore presumably absorbed more than the rabbits.

We can only postulate that PSR block removes some essential element in the complicated sequence of reflex events that is a cough. The essentially expiratory nature of a cough may require the precise activation of abdominal muscles which involves PSRs when the lungs are inflated, as in the inspiration prior to cough (Bishop,1964). However even the inspiratory efforts of our rabbits were impaired by SO_2 block.

Cough provoked by mechanical stimulation of the larynx was reduced but not abolished by SO_2 , this has also been found to be true for chemically induced cough (Hanacek, Widdicombe and Korpas,1980) and the only, rather unsatisfactory, explanation we can tender is that the afferent laryngeal system may provide a more powerful input than the tracheal receptors.

The power of laryngeal reflexes will be vouched for by anyone who has had a crumb of food "go the wrong way"; and the total domination of the process of breathing by the stimulation of what must be only one or two receptor endings demonstrates the danger of looking at the control of breathing as a democratic process dominated by the numerous stretch receptor endings or even more numerous C-fibre endings.

Stimulation of the superior laryngeal nerve, the major afferent nerve of the larynx, demonstrates the global effects of reflexes from this organ outside those of respiratory control. These include bradycardia (Pressmann,and Keleman,1955) and an increase in intestinal movement (Anderson,Landgren,Neil and Zotterman,1950) the evolutionary advantage of which in connection with this organ is difficult to see.

However, it is certain that the reflex activities of the larynx extend beyond the protective cough into a contribution to the control of quiet breathing, and our investigations of this are described in the next section.

Section 2.8 The Larynx

The responses to stimulation of the rapidly adapting receptors (RAR) of the larynx are different to those provoked by stimulation of RAR of the lower airways, as I have described in the previous section of this Thesis.

These differences suggested to us that rapidly adapting receptors in the larynx might have different effects to those in the trachiobronchial tree during quiet breathing.

This lead us to perform the following series of experiments for the following reasons.

The production of coughs by the larynx described in the last section is perhaps its most dramatic and protective reflex influence on breathing, and is the most phylogenetically old (Negus,1945). There are other reflexes which might be interpreted as protective such as the slowing of breathing produced by the irritation of the larynx in the experiments of Boushey, Richardson and Widdicombe (1972). These reflexes might be interpreted as a method of preventing harmful substances being drawn into the bronchial tree by a too rapid airflow, just as a rapid airflow is used in cough to expel harmful substances.

However there is much evidence that the larynx has a role to play in the control of normal quiet breathing e.g. the "expiratory brake" demonstrated by Remmers and Bartlett (1977) and Bartlett, Remmers and Gautier (1977). The larynx is also used in pathological conditions such as pulmonary emphysema to the same effect (Proctor,1964).

It has been known for many years that there is afferent neural activity, modulated by respiration, in the superior laryngeal nerves (SLN) and recurrent laryngeal nerves (RLN) (Aldaya,1936) Part of this activity is rapidly adapting (Sampson and Eyzaguirre,1964).

We had developed a method of specifically stimulating rapidly adapting receptors in the lung (Davies and Roumy,1977) and, because it involved a brief application of intralumenal pressure, which is probably one of the adequate physiological stimuli to the larynx we decided to apply it to that region to investigate if rapidly adapting receptors in the larynx exerted a more subtle influence on breathing than

the frank cough and expiratory efforts elicited by irritant stimuli to the larynx (Boushey, Richardson and Widdicombe,1972).

In anaesthetised rabbits brief (200ms) pulses of negative or positive air pressure were applied to the lumen of the larynx in situ but isolated from the rest of the trachiobronchial tree (Davies and Vizek,1980). The pulses were applied at fixed times from the beginning of inspiration and the duration of the phase of breathing containing the pulse and the following phase were measured as changes in duration of phrenic activity.

Pulses of negative pressure had no effect on the duration of the inspiration (t_I) that contained them. Pulses of positive pressure in the first half of inspiration reduced but did not abolish phrenic activity, which was then prolonged to a greater than control value. The following expiratory duration (t_E) was shortened. Pulses in the second half of inspiration failed to shorten that t_I but the following t_E was still shortened. Discharge from the majority of afferent fibres in the SLN mirrored this pattern with a pause in response to the pulse followed by an extended discharge (Davies and Vizek,1982).

Pulses of positive pressure in expiration shortened that t_E. As with pulses in inspiration negative pressure failed to have an effect.

We filled the larynx with 4%v/w lignocaine hydrochloride which failed to abolish the effects of pulses until it had been in contact for more than 5 minutes .

Pressure within the larynx of the spontaneously breathing intact animal is positive during expiration and negative during inspiration. Bartlett,Jeffery, Sant'Ambrogio and Wise (1976) have demonstrated how the peculiar anatomy of the trachealis muscle, suspended between two points of cartilage, causes a rather symmetrical increase of discharge frequency of slowly adapting receptors for positive or negative pressure about a zero value which exists at a slightly negative pressure. We propose that the insensitivity of the larynx to negative pressure pulses is also due to its peculiar structure, being like a box of rigid plates which resists collapse.

This suggests two things-

1. The receptors involved are situated in the muscles joining the cartilage plates together .

2. These receptors exert their effect more in expiration than inspiration.

The time course of block of the effects of pressure by local anaesthetics suggests that these receptors are located fairly deep in the muscles connecting the plates of cartilage that make up the walls of the larynx. This supports the suggestion of a role as position sensors for the innervation of the laryngeal joints made by Kirchener and Wake (1964).

The types of receptor we recorded were the same as those reported by Boushey, Richardson Widdicombe and Wise (1974). The difference between the shortening of t_E we produced with pulses of pressure and the lengthening produced by electrical stimulation of the SLN (Larrabee and Hodes,1948) did not surprise us as electrocution of the SLN produced the unphysiological situation of a synchronous activation of receptor fibres.

We were initially surprised by the high pressures required to produce the effects we report. However Harding, Johnson and McClelland (1980) reported pressures of $+10cmH_20$ in the larynx of conscious lambs while pressures of $+5cm\ H_20$ produced statistically significant reductions of 10% t_E in our rabbits which were anaesthetised, a state known to depress laryngeal sensitivity (Korpas and Tomori, 1979).

In an earlier section of this Thesis I have described how our experiments with accelerated breathing (Davies and Kohl, 1978 & 1979) lead us to suggest an independent or linked regulation of t_I and t_E depending on the level of RAR activity. Further evidence for this independence comes from these experiments with the larynx, where pulses in the second half of t_I, which did not affect its duration, shortened the subsequent t_E .

Our modification of t_I and t_E in this series of experiments did not affect tidal volume. It must therefore have been by a different mechanism to that described by Clark and von Euler (1972) and Bradley, von Euler, Mattila and Ross (1975) in

which t_I is terminated by PSR activity acting on an "off-switch" whose threshold decreases through the inspiration which it terminates. The work of Clark and Bradley relates to cats. Using figures obtained by D'Angelo and Agostoni (1975) and Trenchard (1977) we would expect the sort of prolongation of t_I we obtained with our pulses to the larynx to allow the "off-switch" threshold to reach a level which would terminate inspiration at about half normal V_T . This was not the case. Tidal volume remained at control values despite changes in t_I suggesting independent control of these variables.

The larynx, although obviously an integral part of the trachiobronchial tree, is demonstrated by us (as others have for many other aspects of this organ) to have important functional differences from the main structure of the tree ; in this case related to its input to the control of quiet breathing.

Section 2.9 Conscious Dogs

It is axiomatic that the ethically imperative use of general anaesthetics in most investigations of the control of breathing in experimental animals makes the understanding of what is happening in the normal unanaesthetised state very difficult. General anaesthetics modify the activity of pulmonary receptors (Coleridge,Coleridge,Luck and Norman, 1964) and the sensitivity of the central neural mechanisms that receive this activity (Severinghause and Larson, 1965). Even if we were to set aside the ethical issue of the use of anaesthetics, which we can not, a subject which is frightened, distressed or in discomfort will obviously not breath with a normal pattern and results obtained under such conditions are of very limited value.

It was with great interest therefore that we became involved in a technique which had been developed by Fishman, Philipson and Nadel (1973) in which both cervical vagosympathetic nerve trunks of three dogs (Candy, Tiffany and Snoopy) were exteriorised into loops of skin, one either side of the neck. This made them accessible for reversible blockade by cooling in conscious stress-free animals.

We had by this time demonstrated some of the effects of highly specifically blocking PSRs with SO_2 in rabbits and also of stimulating RAR with pulses of pressure. I was therefore pleased to be able to investigate the same effects in conscious animals.

In unanaesthetised dogs breathing pattern is irregular, largely as a result of the dog's olfactory investigation of their surroundings by sniffing. It was therefore necessary to occupy their attention by "giving them something to do", this consisted of walking at 3 miles per hour on a motorised treadmill. This seemed to be a congenial occupation after the boredom of sitting in a kennel and the dogs would greet the investigator with enthusiasm when they were collected and jump onto the treadmill un-prompted. Their pattern of breathing was recorded as air-flow by a pneumotachograph attached to a cuffed endothracheal tube inserted in a side-hole tracheostomy which has been prepared several weeks before.

Pulses (0.4s duration $\pm$20cm H_2O) and steps (5 breaths,$\pm$10cm H_2O) of positive and negative air pressure were applied to the lungs via the endotracheal tube while the vagus nerves were intact or blocked by cooling to various known temperatures.

Cooling of the vagi by surrounding them with coils containing circulating alcohol at 7^0C (a temperature which blocks PSR fibre transmission (Franz and Iggo,1968)) caused an increase in t_I and a decrease in t_E as with anaesthetised animals.

The major points of interest in our results were-Whether the vagi were warm or cooled a step of inflation provoked an augmented breath, immediately if the vagi were at body temperature but with a delay of one breath if they were cooled. This pattern of "first breath if warm, second breath if cool" was also seen when negative steps were released.

Pulses of positive pressure in expiration exerted their major effect on the following t_I which was shortened. Pulses of negative pressure immediately initiated inspiration in 4 out of 20 trials if the vagi were warm and in 15 out of 18 trials if it was cool . In anaesthetised dogs there was no immediate initiation of inspiration in 20 trials.

This experimental set-up provided a fascinating new opportunity to investigate breathing in conscious animals. Its shortcomings need, of course, to be considered in interpreting our results. The nature of the block we produced must have been imprecise because the temperature of each vagus probably varied from the outside to the core. Paintal (1966) has shown that high frequency activity in a nerve is blocked at higher temperatures than low frequency activity. This must have obscured the reflexes we provoked . We must therefore look at these changes as trends rather than absolute results.

The changes in pattern produced by cooling the vagi to 7^0 alone were similar to changes seen in anaesthetised animals in response to cooling and other forms of block. This suggests that results obtained under anaesthetic and with these types of block can be applied to the conscious state.

Comparison between conscious and anaesthetised dogs indicate that there is however a differential effect of anaesthetics on the different mechanisms. Application of positive pressure to produce lung inflation during inspiration was stepwise- the result of a solenoid valve opening. This stimulates both SARs and RARs. In the conscious dogs this almost inevitably provoked an augmented breath, not in the inspiration containing the inflation but the next one. In conscious dogs with vagi cooled the augmentation was immediate, in the inspiration containing the inflation, and in anaesthetised dogs there were no augmented breaths provoked in this way. Augmentation is provoked by RAR activity (Davies,1978) and opposed by SAR activity which would operate the inspiratory "off-switch"(Clark and von Euler,1972). On this basis our results can be explained in one of two ways- anaesthesia potentiated the effect of SARs or reduced the effect of RARs. Cooling reduced the effect of PSRs, which allowed augmentation to take place in the first, rather than the second breath. The issue is complicated by the observation that anaesthesia abolishes provoked augmented breaths but potentiates the shortening of t_E during deflation. This suggests there are different sensitivities to RAR activity in the two phases of breathing.

In rabbits we found inflation in inspiration and deflation in expiration to be the most powerful stimuli to RAR. The relative strength of the responses in the present series of experiments suggests that the same is true for conscious dogs . With conscious dogs we found a potentiation of inspiration terminating effects of positive pressure pulses when the vagi were cooled. This paradoxical effect may have been due to the t_I limiting mechanism proposed by Fishman, Philipson and Nadel (1973) but in view of the t_I augmenting role we have demonstrated for RAR it is unlikely that they are involved.

Changes in breathing frequency in our conscious dogs, like those of most species, were brought about by changes in t_E. The control of t_E in these dogs was a balance between extending effects which were blocked by cold (probably PSRs) and terminating effects that could pass a cold block (probably RARs with some C-fibre contribution). Anaesthesia potentiated the t_E extending effect of lung inflation which suggests that experiments with anaesthetised animals may underestimate the contribution made by RARs to the control of breathing in the conscious state.

2.10 Tracheobronchial Responses

Some critics of our previous work investigating pattern of breathing, using SO_2 to block pulmonary stretch receptors (PSR) have expressed concern about the changes in level of arterial CO_2 changes in pattern of breathing may produce, the subsequent changes in airways smooth muscle tone, and the effect of these changes on airways receptors.

I maintain this concern is based on a circular argument, block of PSR produces the change in pattern of breathing and since the receptors are blocked they have no effect that changes in CO_2 can influence.

Never-the-less an opportunity arose to study the tracheobronchial and laryngeal responses to hypercapnia in dogs and to compare these responses to those to histamine and capsaicin.

In previous studies ventilation of anaesthetised animals with increased levels of CO_2 produced contraction of smooth muscle of the airways which was reflected as increases in total lung resistance (Daily, Lambertsen and Schweitzer,1953; Delpierre, Grimaud, Jammes and Mei, 1981_a; Dixon and Brodie,1903; Nadel and Widdicombe,1962), and change in volume of isolated tracheal segments (Green, . and Widdicombe,.1966; Stein and Widdicombe,1975). The time course of these effects seems highly species dependent, with the constriction in dogs being sustained (Green and Widdicombe,1966) while that in cats is followed by a marked bronchodilatation (Delpierre, Grimaud, Jammes and Mei, 1981_a). The significance of these results to our previous work on specific block of PSRs with SO_2 is further thrown into doubt by the observation that although experimental increases in CO_2 concentrations activate nonmyelinated vagal afferents in cats (Delpiere, Grimaud, Jammes and Mei, 1981_b). CO_2 has little action over the physiological range (Coleridge, Coleridge.and Banzett,1978). The ambiguity that exists about this subject is further demonstrated by the fact that although it is generally agreed that inhaling CO_2 rich gas lowers laryngeal resistance in the cat (Bartlett,1979) one study suggests that pulmonary vagotomy had little effect on this response (Bartlett,1980) while another that it is reversed by vagotomy,

suggesting that lung reflexes are involved (Dixon, Szereda-Przestaszewska, Widdicombe and Wise,1974).

We performed this series of experiments in anaesthetised dogs to study the role of vagal reflexes during inhalation of hypercapnic gas mixtures (8% for 30s) on total lung resistance, the volume of an isolated tracheal segment and the resistance of the isolated larynx. In essence these variables were measured with the animal intact, with the right vagus cut below the origin of the recurrent laryngeal nerve and the other nerve cooled to 5^0C, which would block myelinated fibres to the left lung cervical trachea and left larynx (Widdicombe and Nadel,1963). The responses were compared with those produced by injections ($20\mu g.kg^{-1}$)and aerosols (1%) of histamine acid phosphate and injections of capsaicin ($10\mu g.kg^{-1}$). The increases in laryngeal and lung resistance and constriction of the isolated tracheal segment produced by injected histamine could be blocked by vagal cooling to 5^0C, which confirms the previously identified role of RAR (Sellick and Widdicombe,1971). Histamine aerosol produced a similar effect which could not be fully abolished , probably due to a direct action on smooth muscle. Capsaicin produced qualitatively similar effects which, although attributed to C-fibre receptors (Coleridge and Coleridge, 1986) could not be categorically separated from the effect on RARs using our experimental techniques.

The results of these experiments indicate that the laryngeal responses to hypercapnoea depend on vagal integrity, but the tracheobronchial constrictor effect of CO_2 is less affected by vagal denervation.

The changes in CO_2 tensions we needed to use to produce these effects were more than five times greater than any changes seen with administration of SO_2 and that although effects of CO_2 undoubtedly exist, as demonstrated by many other workers, their effects during PSR block by SO_2 are not considerable. If the effects of CO_2 are due to pH changes it would be more important to consider this pH aspect of SO_2 which is a much more acidic gas. We have wondered if this is in fact the mechanism by which SO_2 exerts its effect on PSR.

2.11Vagotomy and phenyl diguanide.

Phenyl diguanide (PDG), administered intravenously, has been used extensively by us and other workers to provoke reflexes attributed to the activation of receptors associated with non-myelinated fibres from the heart lungs and gut.
By accident we received an interesting and salutary lesson concerning this subject which is reported in Davies and Jones (1985) and Davies and Jones (1986).
The respiratory effects of PDG were first investigated by Daws and Mott (1950) who suggested, as many have done since, that the effects of PDG were probably the result of activation of receptors in the lungs, and that these effects were abolished by vagotomy. It has been subsequently demonstrated that these and other non-myelinated fibres in the gut (Paintal,1954), heart and carotid chemoreceptors (Anand and Paintal,1980) can be stimulated by PDG. A large part of the reflex control of pattern of breathing has its afferent arm in the vagus nerves, and since a substantial part of these nerves is made up of non-myelinated fibres the action of PDG is of great interest as a tool to investigate the contribution of these receptors to this reflex control. Other non-myelinated sources of activity should be excluded when attributing changes of pattern of breathing to receptors in the lungs. Species differences also conspire to confuse the picture. Histological investigations indicate there are substantial differences in the ratio of myelinated to non-myelinated fibres in the vagus nerves of different species. Non-myelinated vagal fibres are three times more common than myelinated in the cat (Agostoni,Chinnock,Daly, & Murray,1957) but in the rabbit they are in the minority (Evans and Murray, 1954)
To localise the effect of PDG to pulmonary receptors workers have relied on the observation that intravenous injections of less than 60μg /kg will not stimulate gastrointestinal or aortic receptors and injections of local anaesthetic into the pericardium abolishes the activity of epicardial receptors (Anand and Paintal, 1980) Section of the glossopharyngeal nerves will cut off activity from the carotid chemoreceptors (Chalmers, Korner and White, 1967). This suggests that except for any effect produced by receptors in the carotid region cervical

vagotomy would abolish all responses to injected PDG, if the drug produces its effect solely by activation of peripheral receptors; which is what we, and other investigators had accepted . However Miserocchi, Tripenbach, Mazzarelli, Jaspar and Hazucha (1978) observed that vagotomy did not abolish the respiratory effects of PDG.

In most acute investigations of reflexes from the lungs vagotomy is performed at the end of the experiment (because of its irreversible nature), the stimuli used in the experiment are applied and the absence of response demonstrates any reflex effects were of a pulmonary origin. In most of our experiments, and I suspect in those of others, the stimuli were applied immediately after the vagi were cut. One such stimulus was injected PDG. Up to 1985 we had followed this protocol and found that PDG injected within a minute or two of vagotomy produced no effect on breathing pattern. In an experiment carried out in that year, and not directly related to the interaction of vagotomy and PDG, there was a delay of about 5 minutes after cutting the vagi of an anaesthetised rabbit before PDG could be administered, because of technical problems in preparing the PDG solution. We were surprised to see that the effects of injected PDG were restored under these conditions, presumable because of the delay.

The effects of PDG consisted of an insignificant reduction in t_I and a reduction of t_E to about half its intact control value, whether the vagi were intact, cut (15 minutes after vagotomy) or both the vagi and glossopharyngeal nerves were cut. The absolute value to which t_E was reduced (approximately 0.4s) was the same in each case despite the control value before injection being different in each case (Davies and Jones, 1986).

Intrapericardial xylocaine in its own right reduced t_E and a further reduction was brought about by PDG, this effect was not abolished by vagotomy or cutting the glossopharyngeal nerves provided it was tested not less than 15 minutes after cutting the nerves.

In the absence of intrapericardial xylocaine apnoea occurred as a result of approximately half the injections of PDG, whether the vagus and

glossopharyngeal nerves were cut or intact. In the presence of the local anaesthetic apnoeas never occurred.

In contrast to our (and other's) earlier findings vagotomy did not abolish the acceleration of breathing produced by PDG if a period of about 15 minutes was allowed to elapse after cutting the nerves. We believe that this difference results from the existence of a period of several minutes immediately after vagotomy, even after the vagotomised pattern of breathing has been established, when the rabbit was more or less insensitive to PDG. We are not sure whether this insensitivity is due to a kind of "shock" produced by the barrage of injury potentials synchronously produced by cutting the nerve or the abrupt halt of vagal afferent activity. It may be that cooling and direct current methods of vagal block produce similar effects and Trenchard and Widdicombe (1973) reported a transient perturbation of breathing produced by direct current block which might precede a similar period of insensitivity.

Identification of the origin of the change in pattern of breathing after vagotomy is difficult. Miniglomera exist in the cat (Matsuura,1973) and rat (Martin-Body, Robson and Sinclair,1985) and abdominal neuroepithelial bodies have been identified by Hollinshead(1941) which may have been stimulated. Mechanical electrical and chemical stimuli of the abdominal viscera have all been shown to modify breathing by non-vagal mechanisms. (Frank,1975; Mei,1976; Prabhakar,Marek and Loeschcke,1985). Despite our attempts to avoid stimulation of peripheral sites, by manipulation of dose, section of nerves and use of local anaesthetics stimulation of breathing persisted. This leads us to the suggestion of a central mechanism. Against this suggestion is the fact that PDG is strongly ionised at the pH of plasma and ions of its type do not easily cross the blood-brain barrier (Mayer,Melmon and Gilman,!980), however rapid transport of substances does take place for specific substances at specific sites in the brain (Bloom,1980), and PDG may be one if these.

These experiments did not identify a site of action but they did demonstrate the effects of PDG on breathing persist after vagotomy once a transient refractory period had passed.

Section 2.12 Snoring

In 1987 contact with the Sleep Laboratory at the University Medical School Edinburgh stimulated my interest in pathological obstruction of the upper airways, expressed as snoring and obstructive sleep apnoea, and the question of whether "cot death"- sudden infant death syndrome was related to these conditions. Upper airways obstruction seemed of such general interest that I wrote an article in a magazine subscribed to mainly by women which I titled "Snoring- I suppose you think that's funny". This general article outlined the dangers of upper-airway obstruction during sleep and the causes of snoring . The response to this article was an overwhelming amount of correspondence, mainly in the form of appeals for help from the general public which brought home to me the extraordinary amount of unhappiness this condition causes. At this time my long term colleague Professor John Widdicombe was invited by the pharmaceutical company Anasco GmbH to test a proprietary mixture (Sonarex) which was claimed to prevent snoring. We collaborated in a series of experiments which were reported in two publications (Widdicombe and Davies 1988a and 1988b).

During snoring in man, and the snorer is usually a man, the cross-sectional area of the pharynx is reduced, by a relaxation of the pharyngeal and genioglossus muscles during REM sleep and the negative intraluminal pressures generated during inspiration may be sufficient to cause pharyngeal collapse (Bradley, Brown, Grosmann *et al.*, 1986. Brown, Bradley, Phillipson *et al.*, 1985. Issa and Sullivan, 1984). This of course increases upper airway resistance and the vibration of the tissue around the oropharynx produces the characteristic rattle of snoring. This collapse is resisted by dilator muscles of this area (Olson, Fouke, Hoekje and Strohl, 1988. Van Luteren and Strohl, 1986).

Snoring is inevitably associated with mouth breathing and we suspected that this leads to a drying out of the secretions of the pharynx with a possible increase in their stickiness. A contribution by the adhesion of soft tissue of the upper airway

to closure may have existed as a result of this adhesion in the results obtained by Olson, Wolin and Strohl (1986) and Wilson,Thatch, Brouilette *et al.* (1980), and it is likely that at least part of the benefit, if any, obtained from the mixture we tested (Sonarex) which is insuflated into the pharynx, resulted from increased lubrication of that region.

We decided to devise an animal model of snoring to begin our investigations. Dog owners will know that dogs snore and, as in man, the condition is worse in the old and the fat (Jennett,1984). Brachycephalic dogs (Amis and Kupershoek, 1986) and in particular bulldogs (Hendricks, Kline, Kovalski *et al.* 1987) show sleep disordered patterns of breathing, and bulldogs would have proved an ideal model. However availability and expense precluded bulldogs and we were forced to use the morphologically diametrically opposite breed- the greyhound.

We first studied the conditions necessary to produce snoring in these dogs and what were the changes in upper airway resistance during snoring.

We anaesthetised the dogs with pentobarbitone and placed them supine. We cannulated the trachea just below the larynx in both directions so by connecting the two cannule the dog could breathe normally through its upper airways or we could produce a constant expiratory flow through the upper airway while the dog breathed spontaneously through the caudal cannula. Pressure was measured in the trachea and in the oropharynx to partition resistance from trachea to oropharynx to nose, genioglosuss EMG and the sounds issuing from the airways were recorded.

In 1/3 of dogs snoring occurred spontaneously. The remainder snored when the nostrils were closed with a finger. Pulling the tongue froward lessened snoring. Lateral external pressure on the pharynx wall or flexion of the neck induced snoring . With the nostrils open inspiratory and expiratory resistances were similar and laryngeal resistance 1/6 of total upper airway resistance. With the nose closed inspiratory resistance from the trachea and pharynx was 3-4 times greater than expiratory, and resistance of the oropharynx became up to 15 times greater during inspiration than expiration, presumably due to collapse of a segment of the airway in this region. During snoring, identified by sound, genioglosuss EMG activity

increased markedly. The pressure/flow curves obtained by increasing externally generated flows through the larynx showed a pronounced decrease in resistance in the mid regions of flow which we identified as abrupt changes in position of the soft-palate and epiglottis. During these rearrangements the genioglossus EMG showed bursts of activity which were reminiscent of those reported by other workers in response to mechanical stimulation of the upper-airways (Van Lunteren and Strohl,1986; Widdicombe,1986).

Snoring is in itself an important medico-social problem and is a preclinical condition leading on to more serious states (Lugaresi, Cirignotta, Coccagna, and Piana.1980; Lugaresi, Cirignotta, Coccagna and Montagnna.1984; Norton, Dunn and Haight,1983). As is the case in many other pathophysiological conditions the invasive studies have been carried out on experimental animals and most of the behavioural studies of obstructive sleep apnoea have been in man.

Our model of snoring was deficient in so far as the dogs could in no way be described as obese, nevertheless they snored both in inspiration and expiration. Dictionary definitions of snoring do not state that snoring has to be an inspiratory noise. The pressure/flow loops obtained demonstrated the instability of the upper airway, particularly in inspiration, and although the physics of flow in this region was too formidable for us to make a quantitative comparison with the human condition (Skatrud and Dempsey, 1985) we believe the dog model we used in these studies was sufficiently similar to the human condition to serve.

Our brief was to investigate the mechanism of action, if any, of "Sonarex" a commercially available aqueous mixture of sodium chloride, glycerol, polysorbate 80 and benalkonium chloride. This we did by comparing the effects of insufflating 1ml Sonarex or saline into the oropharynx of our snoring dogs while measuring resistances and recording snoring as previously described.

Both "Sonarex" and saline reduced the airway resistance of the pharynx but we consider the most important difference between the effects of "Sonarex" and the effects of saline to be a greater increase in genoglossus EMG activity and a decrease in the rapid and substantial oscillations in flow and pressure in the

inspiratory phase of flow. The sound of snoring was significantly reduced by 20% with "Sonarex" while saline increased the sound of snoring.

The instructions for use of "Sonarex" are to instil four drops into each nostril (total about 0.5ml) before sleep (Gros,1984;Jennum,1988).

We found that these doses reduced upper airway resistance decreased sound of snoring and increased genioglossus activity. We speculate that many of these effects were due to changes in the adhesiveness of the tissues due to changes in the composition of their mucus covering due to drying out. The opening pressure of the pharynx in experimental animals and dead humans is influenced by tissue adhesiveness (Strohl and Fouke,1985; Wilson, Thatch, Brouilette and Abu-Osba,1980), and the surfactant phosphocholinamin reduces the frequency and intensity of snoring in man (Hoffstein,Mateiko,Haiko and Taylor, 1988).

Increase in genioglossus muscle EMG with "Sonarex" over and above that produced by saline is another potential mechanism of relieving snoring. Reflexes from the upper airways which contract pharyngeal dilator muscles are well established (Olson, Fouke,Hoekje and Strohl, 1988; Vincken,Guilleminault, Silvestri,Cosio and Grassino,1987), and the ingredients of "Sonarex" have properties which could provoke such reflexes; polysorbate 80 is a surfactant which changes the permeability of mucosae (Siegel and Gordon,1986) and removes lipids from cell membranes (Lansdown and Grasso,1972) which is probably the basis of the changes in permeability of membranes seen with these substances (Al-Sourady,Habib and Mohamed, 1985).

What ever the mechanism of action of this proprietary mixture its constituents appear relatively harmless and its actions were superior to saline in reducing snoring

Your author apologises for spending what may appear to be an inordinate amount of space in this Thesis on what might be considered a rather technical subject of little intellectual weight. The response provoked by the popular article I published on this subject convinces me of the widespread distress generated by snoring and the need for further efforts to provide relief.

2.13 Disease models and Species Differences

The activity of the three types of pulmonary receptors recognised as important in control of pattern of breathing in animals is modified by changes in their physical and chemical environment (Bartlett, SantAmbrogio, and Wise, 1976; Bradley and Scheumier, 1977). It is also generally accepted that the environment of pulmonary receptors is changed in lung disease and acute induced pathology (Kohl,and Koller,1980; Mills,Sellick, and Widddicombe, 1973). A possibility that we have considered for some time is that these changes in receptor activity may be the origin of the sensation of dyspnoea which patients with lung diseases complain of most bitterly. Proprioceptive afferent input from the lungs via the vagus nerves with a synapse in the tractus solitarius can ether inhibit or excite the respiratory oscillator of the brain-stem; but evidence that this system, which is powerful in animals, is important man at rest has been elusive. Nevertheless vagal input does become important in driving breathing, particularly frequency of breathing, when the lungs are inflamed, collapsed or waterlogged (Guz 1997). Also, the apparent lack of influence of vagal afferents on healthy quiet breathing in man does not exclude the possibility that they are involved in the transmission of sensation, and in particular the sensation of dyspnoea.

We have produced several animal models of human lung disease, mainly to investigate changes in receptor activity in these conditions, bearing in mind Snider's *caviat* "the usefulness of an experimental model should be judged on how well it answers the specific question it is being used to answer rather than on how well it mimics human disease"(Snider, Lucey and Stone, 1986). Other workers have recorded bulk vagal activity in models of lung disease (Armstrong and Miller, 1980) but this gives little information about the changes in activity of the different types of receptor. Our aim was to record the changes in activity of individual receptors.

Our first experience of modeling human lung disease involved chronic exposure of rabbits to low levels of SO_2 to mimic bronchitis. It was while setting these levels

that we discovered the highly specific effect of SO_2 in blocking pulmonary stretch receptors. Our model was described at the International Conference on Pathophysiology in Prague, 1975.

The first model in which we comprehensively mapped changes in receptor activity was of diffuse pulmonary fibrosis produced by intravenous injections of an emulsion of an olive oil like fatty acid- oleic acid (Davies and Pack,1991). Its action in producing fibrosis seems to be by the droplets of the emulsion blocking pulmonary capillaries and a subsequent chemical stage related to the toxicity of the free fatty acids liberated by the action of pulmonary lipase on the neutral fat (Peitier,1956).

As well as presenting with histological criteria our rabbits showed the reduction in compliance seen in fibrotic patients and the generally reported rapid shallow pattern of breathing which is not chemically mediated (Renzi,Milic-Emilli and Grassino,1982).

In fibrotic rabbits both PSR and RAR were more active than in normals. A shift of balance between these two inputs to control of breathing was demonstrated by changes in the Hering-Breurer reflex which was significantly shorter in the fibrotic than the control rabbits. A significantly greater number of augmented breaths occurred during deflation of the lungs of the fibrotic rabbits compared to normals, and the number of RAR found in the fibrotic rabbits was highly significantly greater. Both the reduction of the Hering Breuer reflex and the increased number of augmented breaths might have been due to the increased RAR activity exerting a Sheringtonian "prepotent" effect over PSR activity.

Our next model was of pulmonary emphysema in rats and rabbits, induced by intratracheal insuflation of elastase or papain. Animal models of emphysema date back over a century, and the early crude attempts at induction reflect the limited and poor understanding of the pathogenesis of the disease at that time (Snider,1992). The use of elastase (Kaplan, Kuhn and Pierce, 1973) produced lesions in the lungs which closely approximate to the morphologic and physiologic features of human emphysema. Of course the pathogenesis is quite different and these models must be viewed as analogues rather than equivalents of the human

disease, which is acceptable as we were interested in the effects of changes in structure on function and our animals showed a 30% increase in the mean linear intercept of their alviolar walls over the control value.

Our initial experiments were carried out on rats (for reasons of economy) and we found that both PSR and RAR activity was increased in the empysematous animals. However there was a greater increase in PSR activity and this was reflected by the changes in pattern of breathing with a slower pattern in the diseased rats with extended t_E and stronger Hering Breuer reflex (Dallak,Davies and Moores,1996). This promotes the suggestion that these receptors might contribute to changes in respiratory drive, and the inefficient patterns of breathing and dyspnoea seen in human emphysema.

This model of emphysema was subsequently studied in rabbits specifically to test the theory that "drive to breathe", in terms of the augmentation of phrenic activity with each inspiration, would be increased in the model. This proved to be the case and our results will be published shortly.

The *bête noire* of physiologists who wish to compare one species of beast to another is that they are different, particularly it seems in their respiration. A number of our studies have exposed, or been bedevilled by these differences. We have found unusual responses of anaesthetized pigs to asphyxia, (Aguggini, Clement and Davies,1979) and attempted to quantify and compare the variability of breathing patterns in different species (Dallak,Xiujie,*et al*,1995).

It is a prosaic observation that small animals breathe with higher frequencies than large ones. Because of this the mechanoreceptors that provide the afferent arm of the reflex control of breathing in these different species have different lengths of time in which to register changed conditions. In other words the adaption of the system must keep up with the rate of breathing. Bartlett and St. John (1979) put forward the very reasonable proposal that the receptors of rapidly breathing species would adapt more rapidly than slowly breathing species to accommodate these differences in rate. When they tested this theory they found that PSR of all the species they tested adapted at the same rate. The difficult question then is how do these different species control their different rates of breathing? We proposed

that the important criterion is the overall adaption rate of the total pulmonary mechanoreceptor system. This would depend on the proportions of rapidly and slowly adapting receptors present. The ratio of RAR to PSR is well documented for rabbits as 1:4 by Roumy and Leitner (1980) and our own experience. Cats have a ratio of 1:10 (Widdicombe,1954b). The situation in rats is less clear. We therefore decided to carry out a careful assessment of the ratio of types of pulmonary mechanoreceptors in rats and found it to be 1RAR to 3PSR. The breath duration of the adult of these three species is also in the ratio of 4:10:3 (Widdicombe,1961). Which supports our suggestion, a modification of that of Bartlett and St..John, that the respiratory frequency of a species is related to the overall adaption rate of all it pulmonary receptors.

Section 2.14 High-frequency ventilation

In a study of the effect of structure of the bronchial tree on lung function (Horsfield, Davies *et al,* 1980) we observed the profound effect of a high frequency oscillation (HFV) on diffusion in a model of the airways. At this time we assumed that this augmentation of diffusion was the reason why patients who receive this treatment to minimise damage to already traumatised lungs (Carlton,Ray,Klain and McCormack, 1980) ceased to make respiratory efforts. However Butler *et al.* (1980) demonstrated that this was not due to hypocapnoea. This apnoea has been demonstrated to be due to increased PSR activity (Sant'Ambrogio and Davenport, 1986). We (Davies and Roumy, 1982) have shown that RAR in the bronchial tree are particularly sensitive to rapid changes in lung volume and that these receptors have reflex effects of a t_E shortening and inspiratory augmenting nature. The presence of tonic phrenic activity in rabbits subjected to HFV (Kohl and Koller, 1984) suggests that HFV may be activating receptors other than PSR , the HFV may be considered as a series of high frequency pulses of inflation and deflation. We combined HFV of rabbits with block of their PSR by SO_2 and static inflation and deflation pressures designed to

increase and decrease the background PSR activity. The incidence of augmented breaths during HFV increased significantly with inflation of the lungs, which may be due to increased efficiency of transmission of the mechanical stimulus. The permissive role of PSR activity in the production of augmented breaths when PSR were blocked, which we have seen before (Davies and Roumy, 1982), was seen again in this study.

It seems clear that HFV stimulates RAR and in a sustained and rapidly reversible way, and could be used as a tool to investigate the cardiovascular and respiratory effects of RAR. It would be interesting to discover if patients undergoing HFV have increased drive to breathe, and (more difficult as they are usually anaesthetised) if they experience increased breathlessness.

In the literature searches which accompanied this work we found reference to the effects of HFV on respiratory mucus clearance in patients; as we had constructed a high frequency reciprocating pump to ventilate our rabbits we decided to use this opportunity to look at the effects of HFV on the properties of sputum. Properties which were not as simple as we had first supposed.

Contradictory results are to be found in the literature on the effects of HFV on mucus clearance in diseases such as cystic fibrosis and chronic bronchitis. Some studies suggest clearance may be improved (George and Geddes,1989) while others have not been able to demonstrate any improvement (Hachenberg, Wendt, Deitmer and Lawin,1987). Bronchial clearance depends on two main features, the nature of the biological system (the bronchial tree of the subject, and the subjects coughing activities) and the nature of what is being cleared- the sputum. It was the physical nature of the sputum, in particular its rheology, we decided to investigate.

This aspect of clearance has not been extensively studied *in vitro* and the methods used frequently were unsuitable for Non-Newtonian liquids whose viscosity depends on shear-rate, and which have elastic as well as viscous properties. There is little wonder King, Phillips, Zidulka and Chang (1984) reported a decrease in

viscosity while Hachenberg,Wendt,Deitmer and Lawin (1987) reported an increase.

The instrument which gives most information about the properties of Non-Newtonian liquids is a parallel-plate viscometer. In this investigation we subjected expectorated sputum to "high-frequency ventilation" allowing the sputum to be oscillated in an artificial trachea for several minutes and then using a parallel-plate viscometer to measure steady sheer flow, creep, and small amplitude oscillatory sheer flow. Oscillation determines the elastic as well as the viscous nature of the liquid while creep gives some idea of the relaxation processes of the fluid.

We found that HFV produced increases in the viscosity and elasticity of the sputum which clearly had time dependent (thixotropic) properties, returning to its original state in a matter of minutes after HFV had ceased.

It should be made clear that our investigation was exclusively of the properties of the sputum and that other mechanisms such as changes in rate of mucus production, rate of ciliary beating may improve clearance. The potential advantages of HFV over the discomfort of daily physiotherapy are such that it should not lightly be discarded .

Section 2.15 Volatile Anaesthetics and Acupuncture

As difficult as the problem of species differences is to the Respiratory Physiologist the problem of anaesthetics is perhaps worse. The ethical and scientific problems of the use or not of anaesthetics are beyond the scope of this thesis and I restrict my report to the investigations we have carried out into the effects of anaesthetics. Some of these have been of a technical nature. We have developed a method of intubation of rabbits, who are notoriously difficult to intubate, which is much less traumatic than other methods I have seen (Davies, Dallak and Moors,1995).

In relation to anaesthetics we have investigated the effects of acupuncture as a respiratory stimulant to reverse respiratory arrest. It is reported that needling of the acupuncture point "Jen Chung" on the "Govenor Vessel 26" which runs through the nasal philtrum acts as a respiratory stimulus. My first, sceptical,

suspicion was that it would be the pain of this procedure which would cause the stimulation.

To see if there was any difference between this and what I supposed would be an equally of not more painful effect we compared the recovery time of sheep anaesthetised with halothane to respiratory arrest when the acupuncture meridian was needled to the same situation when the exposed tibial nerve was needled. The depth of anaesthesia was increased until breathing just ceased, the time for breathing to restart while needling either site was recorded.

Needling Jen Chung was significantly more effective at reducing recovery time than needling the tibial nerve.

The effect of inhaled general anaesthetics on pulmonary receptors has been investigated with the "traditional" volatiles- chloroform, ethyl chloride, trichlorethylene, ethyl ether, divinyl ether, and halothane. The effects of these substances is generally similar and is reviewed by Sant'Ambrogio (1982) to be activation by low concentrations and block by higher. As for so many other stimuli the sensitivity of C-fibres, probably from pulmonary J-receptors, is less than for myelinated fibres. New anaesthetics have come into use which provide a more pleasant experience for the patient. Part of this may be due to a less severe stimulation of airway receptors, and Nishino, Anderson, and Sant'Ambrogio (1994) have investigated the responses of trachiobronchial receptors to halothane, enflurane and isoflurane. We have compared the effect of sevoflurane, a modern volatile anaesthetic which produces markedly less tachypnoea than other volatiles with halothane and found it has significantly less effect than halothane on pulmonary afferent activity (Moores,Davies and Dallak, 1998).

During this series of experiments we had the opportunity to obtain a perfluorcarbon (PF5080, 3M Industrial Chemical Products) which is used in liquid ventilation to support injured lungs (Hirschel, Pranikoff,Gauger *et al.* 1995). We were surprised to find that filling the lungs of anaesthetised rabbits to FRC with this volatile hydrocarbon produced no detectable change in activity after it had been removed. (Kelly, Moores,Stenson, Davies and Drummond,1999)

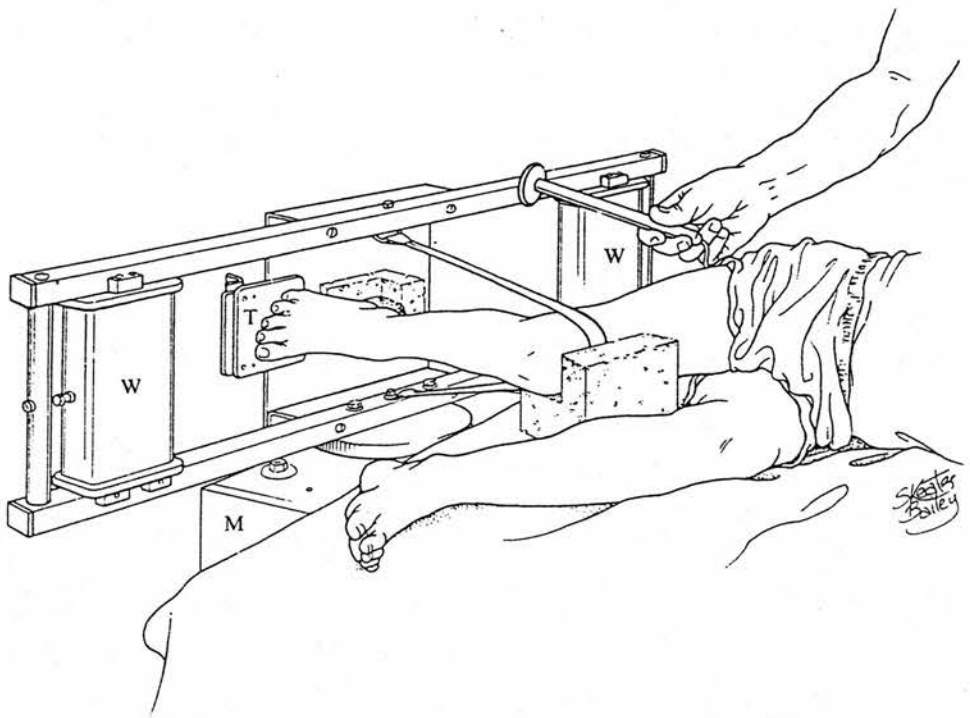

Fig. 1. The apparatus used. The instrument is designed for movements of the foot in the horizontal plane to avoid the confusion which would otherwise result from the effects of gravity. 'M' is the motor housing, 'W' indicates the rectangular canisters into which lead has been cast. 'T' is the plate fixed to the transducer (load cell).

Section 3.SUMMARY OF STUDIES OF HUMAN MOVEMENT AND TECHNICAL CONTRIBUTIONS

Section 3.1 Human Movement and Tremor

I have made minor excursions into the study of human movement accompanied by colleagues at the University of Edinburgh. We were initially interested in the generally applied clinical test of the tendon-tap. This test is a rapid and non invasive method of obtaining information about muscle properties and has been used to demonstrate that muscle relaxation time is increased in hypothyroidism and decreased in hyperthyroidism (Marsden, Meadows and Lange,1970) as well as identifying changes in properties in diabetes (Bearwood and Schumacher, 1964), obesity (Burt and Stunkard, 1964) and cerebral palsy (Wall, Umlauf and Geppert, 1964). Many investigations have been flawed by their instrumentation. Most have used isotonic contractions in which the swing of the limb (usually the lower leg) has been influenced by the weight of the foot the position of the centre of gravity, the inertia about the ankle and the passive elasticity of the dorsiflexors. Many of these problems are removed by using isometric contractions. One problem that is not removed by using this form of contraction is the influence of the exact placement of the limb and the degree of relaxation on the initial loading of the muscle under test. We overcame this with an instrument of unique design which consisted of a large, but freely moving mass to which the foot is attached. The load on the muscle is applied by rotating this mass with a printed circuit motor whose axis of rotation was at the ankle- the torque applied by this motor determined the initial tension in the muscle and when the tendon was tapped the contraction of the muscle was over before the large mass started to move.

(See Fig 1 opposite)

By virtue of these arrangements the contractions were isometric, starting from a predetermined preload.

We compared the forces and time course of events in male and female students and in elderly men and women when soleus was stimulated by a tendon tap. Soleus muscle is composed predominantly of slow twitch fibres and we found

there was no evidence of slowing of contraction in the older people. There was, as would be expected a reduction in peak torque in the older population, and in both populations the women had significantly longer half-relaxation times.
The most likely cause of this is the smaller diameter of fibre found in women (Lindman,Eriksson and Thornell,1991). These differences have implications for the interpretation of data from clinical studies and at a cellular level may be due to differences in rate of reuptake of calcium.
We used our apparatus to compare the parameters of quadriceps contractions of highly muscular members of an University Rowing Crew to those of ordinary Medical Students. The timing of events of the rowers muscles was no different from "normals"(Walsh,Davies and Powers, 1997).

We have studied human tremor under a number of conditions. For example there is some dispute as to whether coffee or smoking cigarettes causes tremor. Using an accelerometer attached to the subjects finger, which was held horizontal we found smokers had no more tremor than non-smokers but that drinking two cups of instant coffee produced a significant increase in finger tremor (Davies,Elton, Powers *et al.*,1990).

It might not surprise the reader to learn the greatest degree of tremor we have recorded (unpublished observation) was in a subject about to undertake a "bunge-jump" from a 70m tower.

Section 3.2 Technical Contributions

The execution of original research frequently requires the development of new techniques or instruments to produce the effects or measurements the investigator requires. It seems in my investigations that this has been particularly true and I outline below a few of the techniques and devices whose construction I have published in the hope they would be useful to other investigators.

Lung morphometry

My interest in measurement of gas transit times in the mammalian lung was promoted by construction of hollow casts of the bronchial tree. This was possible by adapting the engineering technique of "lost wax" casting, and adapting it to a method of electroplating which produced accurate hollow casts. The technique is reported in Timbrell, Bevan, Davies *et al* (1970).

To measure gas transit times required a method of accurately and very quickly detecting the passage of a gas front through the small tubes that comprise the bronchial tree. In the 1970s respiratory mass-spectrometers were rather unreliable, cumbersome and above all expensive. I therefore adapted a commercial leak detector, used in high-vacuum systems, to detect the arrival of a gas front "tagged" with minute amounts of halogenated hydrocarbons. This instrument and the technique of using it is described in Davies (1971 &1972).

Neurophysiology

The much used technique of blocking Pulmonary Stretch Receptors with gaseous sulphur dioxide in air requires the sampling of the mixture to ensure it contains the correct concentration of SO_2 (200p.p.m.). This was originally done using a commercial colourimetric method (Drager Normoair) which involved repeated sampling with detector tubes that could only be used once. This was expensive and so a device was developed which relied on the change in conductivity of a hydrogen peroxide solution to measure the concentration of SO_2 in air. (Wise and Davies, 1976).

Part of these studies involved integrating nerve activity, frequently the bulk discharge of the phrenic nerve. At this time personal computers were not available to carry out this task and instability and drift of solid state integrators made them unreliable and workers relied on "leaky" integrators to provide a workable solution. We developed a "true" integrator which summated all the signal presented to it and re-set automatically at the end of each breath. This is described in Davies and Wise (1978).

Neurophysiological recording from "single" nerve fibres is as much an art as a science and I am sure other workers have been confronted with the problem of difficult or inferior quality "singles" whose shortcomings may be due to a fault in the electronic instrumentation or alternatively an actual absence of activity in the nerve, due to damage or some other factor. What is required is an absolutely reliable action potential at the recording electrodes against which the recording apparatus can be tested. To provide this we constructed a tiny signal generator which provides a "Pseudospike" to the recording electrodes. (Davies and Davies , 1999). This has proved a great time saving device.

I have already described two instruments (measuring gas transit time and SO_2 concentrations) which we had to devise, rather than use commercial alternatives, for reasons of economy. On many occasions we wished to administer aerosols and rather than purchase a commercial generator we have described how an ultrasonic domestic humidifier may be modified to serve the purpose (Davies and Pirie ,1995).

We have always been concerned with the animal welfare problem of intubating rabbits whose anatomy makes this procedure difficult. We have developed a new method which we think minimises the trauma of this procedure. (Davies,Dallak and Moores,1996).

Human movement

In my association with Dr.E.G. Walsh on our studies of human movement we have developed new methods of measuring isometric tension (Walsh, Wright, Davies *et al*, 1993), and an automated method of measuring knee jerk(Walsh,Davies and Powers, 1995).

To measure slight respiratory movements of air we have produced a highly sensitive detector based on the "fluid switch principle" (Ponting, Heusch and Davies, 1999).

My investigations of Lung Morphometry were carried out at the Medical Research Council Pneumoconiosis Unit, Llandough. Control of Breathing was investigated at St.Georges Hospital Medical School London, the University of Texas, Galveston; Cardiovascular Research Unit San Francisco and the University of Edinburgh where my work on Human Movement was carried out. I thank my friends at these institutions for the pleasure of their collaboration.

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Section 6: Candidate's Publications.

Copies of their original form (In chronological order).

Book chapters.

Books.

Publication 1.

Timbrell, V., Bevan, N.E.,Davies,A.S. &
Munday, D.(1970)

Hollow casts of lungs for experimental purposes.

Nature,Vol. 225, No., 5227, pp.97-8.

We consider these results to be important because mice but not hamsters were available as the gametocyte carrier and because another anopheline species, not related to the natural vector, has been demonstrated as an experimental vector of rodent malaria.

We thank Professor Yoeli for the strain of *P. berghei* and for his encouragement. We also thank Privates Gulley and Green and Corporal Kreutzmann of the 1 Malaria Research Laboratory, RAAMC, and Mr J. C. Walker of the School of Public Health and Tropical Medicine for technical assistance.

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Hollow Casts of Lungs for Experimental Purposes

SEVERAL techniques have been described for producing solid casts of human and animal respiratory tracts in wax[1], Wood's metal[2] and 'Vinylite'[3]. Made with care, these casts keep the fine detail of the airways extending as far as the respiratory bronchioles. But because the casts are "negatives", in that they represent the airspaces rather than the airway walls, their use has been limited to demonstrations of anatomical features and pathological changes[4]. Hollow positive casts for use in airflow studies have been made from these negatives by conventional casting techniques; some made in resin[5,6] have contained

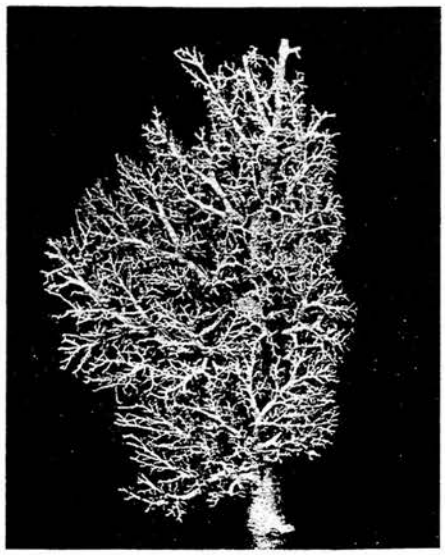

Fig. 1. Hollow cast of a pig's lung.

the airways from the trachea as far as the segmental bronchi. This article describes a technique for producing hollow casts which keep the fine detail obtainable in the solid models; a hollow cast of a pig's lung is shown in Fig. 1. Preservation of detail of the respiratory bronchioles greatly increases the value of such casts for the study of airflow and of particle deposition. The casts are made by spraying a negative wax cast with colloidal silver and electroplating onto the conducting layer a firm structure from which the wax is then removed.

The lungs may be used fresh or after storing in deep freeze at −20° C. To avoid risk of tearing the pleura, stored specimens must not be manipulated until completely thawed. The lungs are treated separately, the trachea being cut at the hilum leaving the main bronchus as long as possible. The bronchus is cannulated with a plastic tube and tied in position. The lung is warmed in a water bath at about 37° C for an hour to make inflation easier and more uniform than if the lung is at room temperature. It is smeared with a dilute gelatine solution to preserve the flexibility of the pleura and then inflated in an artificial thorax as used by Weibel and Vidone[7]. This apparatus consists of a glass cylinder with a 'Perspex' lid fitted with a metal tube from which the lung is suspended by the cannula. The cylinder is evacuated to a pressure of 15 cm of water below atmospheric pressure, resulting in air being drawn through the pleura, which is permeable. In these conditions the lung assumes normal inflation contours.

Warm air is drawn through the lung for 2–3 h. This warming and drying of the lung enable the wax to penetrate to the fine airways. One to two hundred ml. of wax is required for a human or a pig's lung. Paraffin wax with a melting point of 60° C is suitable. The wax is heated to 80° C, 0·25 per cent w/v Oil Red O dye is added to colour it, and 5 per cent v/v iodized poppy seed oil (Lipoidal Ultra Fluid Iodized Oil) added to make it radio-opaque, and it is then poured into the expanded lung. The cylinder is kept evacuated for 1 h while the wax is setting. Storing the lung before use tends to make the pleura more permeable and penetration of the wax more complete.

After the lung has been left for several hours to allow the wax to harden, it is removed and X-rayed in three aspects. Examination of the X-ray plates shows if the wax has penetrated all the airways. If penetration has been satisfactory the tissue is digested in a bath of concentrated hydrochloric acid for 2–3 days: to avoid damage to the fine airways the bath should be deep enough so that the exposed cast will finally float to the surface, free from the bottom of the vessel. The cast is then washed with water and a copper tube substituted for the plastic cannula. It is again compared with the X-ray plates to see if damage has occurred during the acid digestion. Several coats of colloidal silver (Type 915, Acheson Colloids Co.) are applied to the dry cast using an atomizer spray. The red dye in the wax facilitates detection of thin areas of coating which can be covered either by spraying or by using a camel-hair brush.

In most studies the ends of the airways in the finished hollow cast need to be open so that air or aerosol can be blown or sucked through via the main bronchus. This is achieved with a tiny wire ring to apply blobs of molten paraffin wax to the tips of the airways in the coated solid cast before the electroplating. Dye is put into this wax to ensure that all terminal airways are treated. The distance the ring is taken along the airway determines the final terminating diameter. Silver is preferable to copper as the plating metal, as the deposition rate is higher and the deposit more uniform. A satisfactory electrolyte in g/l. is 24 silver nitrate, 35 sodium cyanide, 20 sodium carbonate and 6 sodium hydroxide.

The model is suspended in the plating bath from a spindle which rotates once every 10 min to assist uniform plating: the electrolyte is gently stirred. To prevent marked preferential deposition on the peripheral regions the bath needs to be large enough for adequate separation to be provided between the model and the anode. For a satisfactory plating rate, the surface area of the anode needs to be at least equal to that of the model (200 sq cm for a human or pig lung). A low initial current (0·5 mA/sq cm) is required for good adhesion. This is increased progressively to 3 mA/sq cm after 3 h. The model is examined periodically and areas where plating has not occurred are resprayed and plating continued. Models are normally plated to a thickness of 0·01 inch (30 h plating) and the bronchus and main airways further strengthened by brushing on a coat of epoxy resin. The wax is removed from the cast by heating to 80° C in an oven for an hour. Residues are removed by blowing a jet of air into the bronchus after the cast has been heated to 100° C. Smoke is blown into the cast to detect leaks, which are repaired with epoxy resin.

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Windborne Dispersal of Foot and Mouth Virus

Several papers[1–7] have suggested that wind-carried foot and mouth virus (FMV) had helped to spread the disease during the 1967 epidemic. Most of the papers

Publication 2.

Davies,A.S. 1971.

Measurement of gas transit times in excised lungs and hollow casts.

J. Physiol. 213, 15-17p.

The assessment of pulmonary surfactant in rat lungs

By M. M. Collins, S. Ghobrial and M. McDermott. *M.R.C. Pneumoconiosis Research Unit, Llandough Hospital, Penarth, Glamorgan*

Measurement of gas transit times in excised lungs and hollow lung casts

By A. S. Davies. *Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, Glamorgan*

Realistic modelling of gas flow mechanisms in normal and diseased lungs requires information on the time for inspired gas to pass through the bronchial tree to small bronchioles (transit time). In this demonstration we show how transit times from the carina to points within the bronchial tree may be measured in excised lungs at approximately equal degree of inflation, and hollow casts of lung airways produced by the method of Timbrell, Bevan, Davies & Munday (1970).

The lung or cast is ventilated by a positive pressure sine wave pump with a stroke of 250 ml. and a frequency of 12 cyc/min. At a predetermined point near the start of the respiratory cycle a bolus of halogenated hydrocarbon gas (dichlorodifluoromethane) is injected into the main airstream. The arrival of halogenated hydrocarbon within the bronchial tree is detected by a halogen-sensitive diode, through which a vacuum pump draws a small continuous sample of gas via a catheter implanted at the chosen point. A second diode, sampling gas near the carina, acts as a fixed reference point. The time interval between the signals from the two diodes (after allowing for delays due to the sampling catheters) is the transit time between the two sampling points.

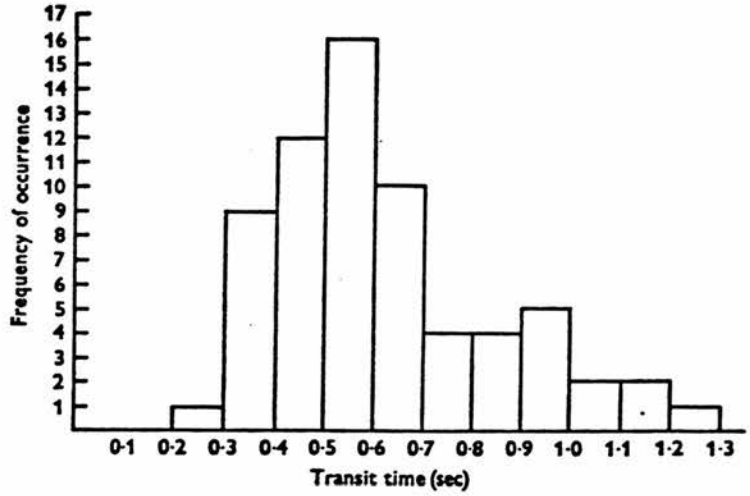

Fig. 1. Frequency distribution of 66 transit times.

In the hollow cast, the catheter samples from a succession of small holes drilled at 1 cm intervals in thecast wall. With excised lungs a retrograde catheter of the type described by Macklem & Mead (1967) enables samples to be drawn from airways of the same diameter but situated in different regions of the lung.

It is found that a wide range of transit times exists for gas to reach bronchioles of the same diameter but situated in different regions of the lung (Fig. 1). This is probably the result of a range of bronchial path lengths, as measurements on the hollow casts show transit time to be a function of the distance of the sampling point from the carina.

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*Publication*3.

Davies,A.S.(1971)

Gas transit times in the porcine lung.

J. Physiol. 222, 76-77p.

PHYSIOLOGICAL SOCIETY, DECEMBER 1971

Gas transit times in the porcine lung

By A. S. Davies. *Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, Glamorgan*

A method has been described of measuring the time taken by a front of gas to pass through the bronchial tree to small airways, i.e. transit time (Davies, 1971). We now present results of measurements made by this method.

Cumming (1967) has suggested that if gaseous diffusion within the terminal airways of the lung is complete the total inefficiency of gas mixing will be determined by the factors which affect transit times. These include the asymmetrical structure of the bronchial tree (which results in airways of the same diameter existing at different distances from the carina), variations in compliance, and the gradient of pleural pressure over the surface of the lung. The results presented here were obtained with hollow casts of the bronchial tree, excised lungs, and excised lungs subjected to a gradient of positive pressure over their surface. In this way the factors affecting transit times could be studied in isolation, and their respective influence noted.

The effect of the asymmetrical structure of the bronchial tree on the distribution of transit times was investigated using hollow casts (Timbrell, Bevan, Davies & Munday, 1970). Transit times from the carina to points at 1 cm intervals along selected bronchial pathways were measured using dichlorodifluoromethane to 'label' the advancing front and sampling the gas through 1 mm diam. holes drilled in the cast wall. There was a linear relationship between transit time to an airway of a particular diameter and its distance from the carina. This was subsequently confirmed in excised lungs using impacted retrograde catheters of 0·5, 0·24, 0·15 and 0·12 cm diameter (cf. Macklem & Mead, 1967).

Using the latter technique the distribution of transit times was found to vary inversely with the diameter of the airways. The measurements were repeated with the lungs suspended in a fluidized bed of expanded polystyrene beads (Schroter & Sudlow, 1969) to simulate a gradient of pleural pressure over their surface. With the lung in different positions transit times were found to be increased to the dependent regions.

These measurements bear out the suggestion promoted by morphometric studies that the asymmetrical structure of the bronchial tree produces the basic form of uneven distribution of ventilation in the lung. Upon this the effects of compliance and a gradient of pleural pressure are superimposed.

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Timbrell, V., Bevan, N. E., Davies, A. S. & Munday, D. E. (1970). *Nature, Lond.* **225**, 97–98.

Publication 4.

Davies,A.(1972)

A study of gas transit times through the bronchial tree.

Ph.D. Thesis. University of London.

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ABSTRACT OF SUBMISSION FOR HIGHER DEGREE

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Degree in view... Ph.D..... *Date*... 1972.....

Title of submission... A Study of Gas transit times Through the Bronchial Tree.....

The thesis describes a method of measuring the time taken by a front of gas to pass from the carina to individual small airways in hollow casts of the bronchial tree and excised lungs, i.e. transit time.
The techniques involved included a method of producing hollow casts of the bronchial tree in previously unobtainable detail. The modification of an industrial instrument to provide a rapid detector of gas fronts is described, together with a sampling system that caused minimal disturbance to gas distribution in the lung. A new method of producing an experimental gradient of pleural pressure over excised lungs is described.
Using these techniques measurements in hollow casts showed how a front of gas advanced in the asymmetrical system of branching tubes that is the lung airways. A large reduction in the linear velocity of the advancing front was demonstrated.
In excised lungs a distribution of transit times was found to airways of the same diameter but situated in different regions of the lung. The relationship between transit time to a point and its distance from the carina was similar in casts and excised lungs, indicating that regional differences in compliance, found only in the lungs, were not sufficient to affect the relationship.
A gradient of pleural pressure over the surface of the lung altered the relative compliance of the upper and lower regions of the lung in such a way as to cause significant increases in transit time to the dependent regions when the lung was in the upright position. A comparison of the distributions of transit time in the rigid casts, the excised lungs and the lungs subjected to a gradient of pleural pressure demonstrate the pathway length to be the most significant feature determining transit time and the imposition of a gradient of pleural pressure exaggerates the effect of pathway length.
These results represent a new method of describing the uneven ventilation of the lung . The method allows the effect of the asymmetrical structure of the bronchial tree to be separated from the effects of compliance and pleural pressure. It demonstrates that the asymmetry of the bronchial tree results in a basic inequality of ventilation. On this the effects of compliance, and to a much greater extent, the presence of a gradient of pleural pressure are superimposed.
The results suggest that the structure of the bronchial tree may act as a "molecular filter" and cause separation of the components of a mixture of gasses. This possibility is to be investigated.

Use this side only

Publication 5.

Davies,A.(1973)

Evolution of bronchial casts.

Medical History, 17, 386-91.

News, Notes and Queries

THE EVOLUTION OF BRONCHIAL CASTS

The mammalian lung provides a system of airways in which inspired air is brought into intimate contact with the blood. As well as carrying out this essential physiological function, the hollow structure of the lung has enabled investigators to produce casts of the airways, the study of which has helped in the understanding of air movements within the lung.

The dependence of higher forms of life on air must have been obvious to the ancients. Anaximenes of Miletus (born *c.* 570 B.C.) stated that air or 'pneuma' (literally 'breath') was essential to life. The function of inspired air was open to some speculation. Plato (428–345 B.C.) stated 'as the heart might easily be raised to too high a temperature by hurtful irritation, the genii placed the lungs in its neighbourhood, which adhere to it and fill the cavity of the thorax, in order that their air vessels might moderate the great heat of that organ, and reduce the vessels to an exact obedience'.

The indefatigable Galen (A.D. 130–199) also interested himself in the function of respiration. It may be that he was the first to have insight into its true nature for he compares it with a lamp burning in a gourd, 'When an animal inspires it is, I think, similar to a perforated gourd, but when respiration is prevented at the appropriate place on the trachea, you may compare it to a gourd unperforated and everywhere closed'.

It seems that these ancient investigators did not concern themselves with the fine structure of the lungs and bronchial tree, and the record of their observations was restricted to simple drawings and woodcuts. But with the fifteenth century came a new interest in art and science which heralded the Renaissance. Anatomy derived great benefit from the more liberal ideas of the period, and the nature of the human body, demonstrated by new techniques, began to be accurately represented by the great artists of the time. One such technique was the use of casts to visualize hollow structures in the human body. Leonardo da Vinci, that 'modern biologist in the guise of a medieval artist' (F. J. Cole) injected melted wax into the ventricles of the brain to demonstrate their structure. His method, while subject to a number of defects, corrected misconceptions evident from his earlier drawings, and provides the first steps in the technique used in this laboratory to produce hollow casts of the bronchial tree nearly 500 years later.

While it appears that Leonardo planned to produce a hollow cast of the cavity of the aorta in its ascending portion, he never applied the technique to the bronchial tree. His ideas on the function of the lungs, which he considered a single organ, are largely due to Galen. About the parenchyma Leonardo writes: 'The substance of the lung is dilatable and extensible. It lies between the ramifications of the trachea in order that those ramifications may not be displaced from their position, and this substance is interposed between these ramifications and the ribs of the chest to act as a soft covering'.

Not surprisingly Leonardo assumed that the total area of cross-section of the ultimate branches of the bronchial tree was equal to the cross-sectional area of the trachea, a misconception not completely corrected until very recently by the use of highly

detailed casts of the bronchial tree and sophisticated mathematical analysis of the nature of airway branching.

The inability of Leonardo and subsequent workers to demonstrate fully the structure of the bronchial tree may be explained in the words of Sir Russell Brock who says: 'Only by a complementary use of bronchial casts or dissections, and of specimens in which the segments are injected separately, can the full story be learned and depicted'.

The use of solidifying injection material, which was a pre-requisite for the production of casts of the bronchial tree, sank into abeyance after Leonardo until it was reintroduced by Jan Swammerdam (1637–1680). He injected a number of organs with wax, though there is no record of his attempting to preserve the bronchial tree in this way. Unfortunately all trace of Swammerdam's preparations have now been lost.

Following Swammerdam's example many materials were tried to discover a suitable solidifying injection, but it was Guillaume Hamberg (1652–1715) who first introduced the injection of alloys. In the *Mémoires de l'Académie des Sciences* (1699) he describes his method of injecting a mixture of equal parts of lead, tin and bismuth which he found would remain liquid at temperatures 'less than was required to scorch paper'. This technique seems to have stimulated an interest in the bronchial tree, for a number of workers, including Govert Bidloo (1649–1713) and William Cowper (1666–1709), attempted to produce metal casts of the air cavities of the lungs. Bidloo claimed that his casts were produced by the injection of molten bismuth, but as Tompsett points out the melting point of bismuth (212°C) is much too high for this to be possible. He probably used an alloy or an amalgam of bismuth. These materials are very brittle, which may explain why none of these preparations has survived. An interesting offshoot of the injection of metals into the lungs was the introduction by Marcello Malpighi (1628–1694), the founder of microscopical anatomy, of mercury injections with which he investigated the structure of the lungs.

The anatomists of the eighteenth century carried these techniques to a high degree of perfection, and while most of their delicate wax corrosion casts have now perished, a record of their existence still remains. In the Royal College of Surgeons of England, there is a portrait of William Hunter (1718–1783) which includes a corrosion cast of the human heart and lungs. Notable among the workers of this period is Thomas Pole who, in 1790, published his textbook of anatomical techniques. His book not only demonstrates his command of anatomical techniques but also offers good advice on the personal qualities necessary for this exacting work. 'In making injections, the principal ingredients, and the first to be obtained, are time and patience, and not less so, a uniform fortitude against disappointments; for it will not infrequently happen that, with the greatest care, a most promising preparation will be instantaneously destroyed by some trivial accident'.

His advice on the 'defence' of casts of the bronchial tree is most sound and especially applicable to casts made of fragile materials such as wax.

> Preparations injected for the purpose of corrosion should always be carefully handled, lest the injection be incautiously broke, which, in their finished state having no support from the surrounding vessels, will fall to pieces; this would be an unpleasing circumstance, after everything else had been successfully conducted. . . .
> These preparations require great care and much time to complete them, and when finished, are of all others most liable to be demolished by trivial accidents; it is therefore expedient to defend

> them as much as possible from possible injuries . . . for persons who have not made them are not always satisfied with looking, but every now and then trying their strength by the finger, at the expense of destroying its most beautiful parts . . . .

Despite the perfection of injection techniques attained by the end of the eighteenth century, there were no significant advances in the subject of bronchial anatomy for a hundred years. In 1880, however, Theodor Aeby published his treatise *Der Bronchialbaum der Saugethiere und des Menschen, nebst Bemerkungen über den Bronchialbaum der Vögel und Reptilien.* In this monument of patient research Aeby carried out measurements on metallic casts obtained from some forty-eight individual species, belonging to fourteen mammalian families. In the section devoted to the human bronchial tree metallic casts were derived from a series of individuals of different sexes, and at various ages including the later foetal months. Unfortunately Aeby's determinations were made exclusively on metallic casts, and while these are capable of great detail they are also liable to serious errors.

It may have been because of these artifacts that he managed to see in the human lung a bronchial stem from which the bronchial tree arose by monopodic branching. Aeby's prestige was so great that his views were generally adopted for some fifty years in spite of the challenge put forward by William Ewart (1848–1929) in *The Bronchi and Pulmonary Blood Vessels* (1889). Ewart confined his investigations to human lungs from which he prepared solid casts of the bronchial tree in low melting point alloy. He injected the isolated lung in the belief that it was Aeby's practice of injecting the lung within the thorax which had lead him astray. From consideration of his casts he concluded that the shape of the bronchial tree conforms to the shape of the thoracic cavity, pointing out for the first time how misleading comparisons between the bronchial tree of man and other mammals could be. Ewart also pointed out that the lung could be subdivided into a number of separate anatomical units. He says 'Within each lung large groups of lobules are kept in practical isolation from each other as regards their air supply. Each of these sub-lobar groups may be considered as forming separate respiratory districts'. Ewart's concept of segmental anatomy remained unexploited until after his death when Kramer and Glass in 1932 coined the phrase 'bronchiopulmonary segment' to describe the 'respiratory districts' seen by Ewart in his metal casts some fifty years earlier.

The predecessors of the present-day plastic casts are the cellulose and vinyl resin (Vinylite) casts of Liebow, Hales, Lindshog and Bloomer (1947). Vinylite itself has now been displaced by the cold-setting synthetic unsaturated polyester resins, such as Marco resin. These materials make it possible to produce beautiful, rigid, coloured casts of anatomical cavities. Notable in the use of resins is D. H. Tompsett, who in 1952, described the method of producing resin casts which has been used extensively by morphometrists and other workers wishing to produce solid casts of the bronchial tree up to the present day. Tompsett's original method involved filling the bronchial tree with gelatin and submerging the whole lung in warm water. The gelatin was then displaced from the airways by resin run in through the trachea. When the desired degree of penetration had been attained the gelatin was solidified by pouring iced water over the lung. This prevented further penetration of the resin which was allowed to set. The tissue was then digested away leaving the plastic cast. An alternative method has

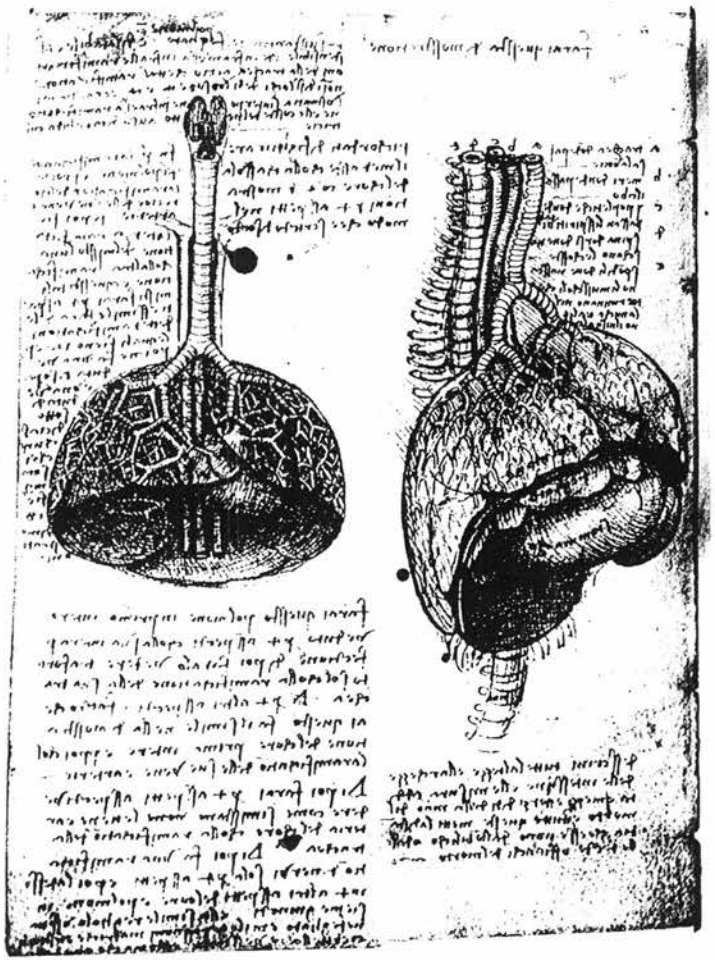

Figure 1
Drawing of lungs and trachea by Leonardo da Vinci.

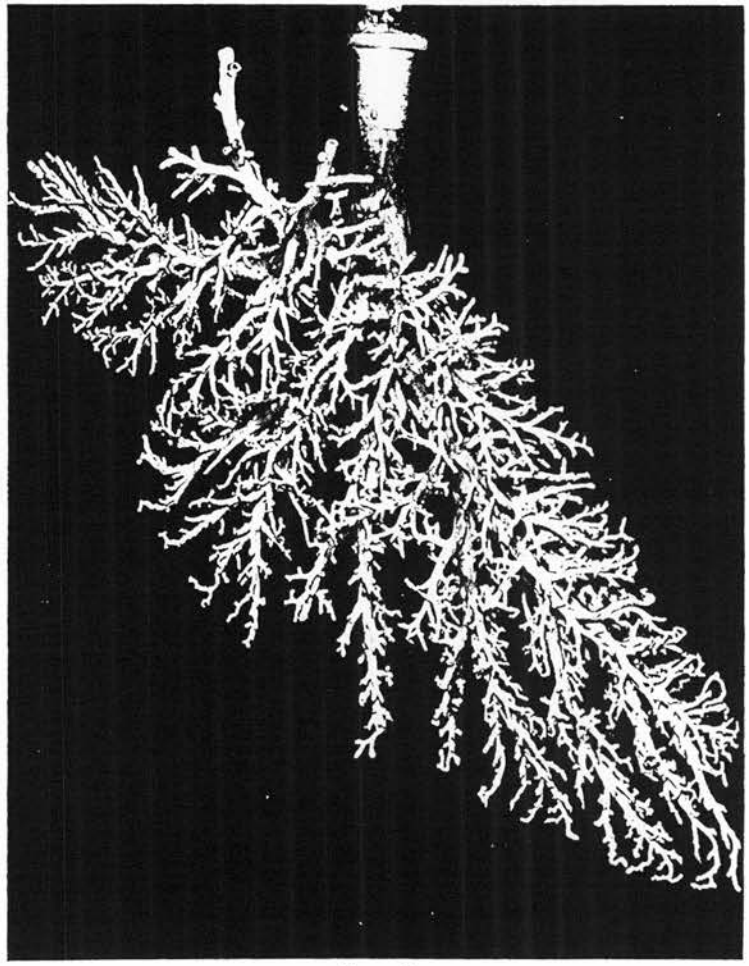

Figure 2
Hollow cast of pig bronchial tree. Produced by the author

been described by Rahn and Ross (1957) in which the lungs are dried and fixed in an inflated state by a continuous flow of compressed air through the trachea for two to three days. A polystyrene plastic is poured into the trachea and cured by heating the whole at 80°C for three to six hours.

Solid casts of the air spaces of the bronchial tree have aided, and deceived, anatomists in their investigations of the human lung. An interesting application is the use of measurements made on casts to develop mathematical models of the bronchial tree. These models are then used to predict the nature of gas flow in the lung. No discussion of the use of lung casts for morphometric purposes would be complete without reference to Weibel. His monograph on lung morphometry marked a new era in the subject, and his measurements have been the basis of calculations by many workers of the nature of ventilation in the human lung. Weibel measured all the limbs of a resin cast down to the fifth order of branching, and a sample of the limbs down to the tenth order of branching. From the tenth order of branching to the smaller structures, which he investigated by histological methods, Weibel assumed regular dichotomy and predicted the missing measurements. Because of this the effect of asymmetry in bronchial anatomy was excluded. A number of workers (Rohrer, Findeisen, Hilding and Hilding) have similarly treated the bronchial tree as a symmetrical system. These models demonstrate the idealized state in which all alveoli are equidistant from the carina. Ross (1957) made exhaustive measurements on resin casts of dog lungs and demonstrated that the bronchial tree is in fact an asymmetrical system. Horsfield and Cumming (1968) carried out similar measurements on solid casts of the human bronchial tree. They went on to calculate that the asymmetry they had demonstrated would affect the distribution of ventilation in the lung.

An interesting development in the field of casts of the bronchial tree has been the advent of hollow casts. While these have only been produced in the past twenty years, it is interesting to note that Leonardo proposed to make such a cast of the ascending aorta some 500 years ago. The hollow casts are an obvious step beyond the solid models. For while with solid models only morphometric measurements can be made, and conditions of air flow extrapolated from these, hollow casts are susceptible to direct experimental investigation.

A precursor of the use of hollow casts to investigate flow is seen in the work of E. A. Gaensler, J. B. Maloney and V. O. Bjork (1952). These workers investigated the nature of gas flow in tubes of the same diameter as the human trachea. They discovered that turbulence occurred at much lower flow rates than Rohrer (1915) had calculated from morphometric studies. They established that turbulence probably existed in the bronchial tree at all but the lowest flow rates. The first true hollow cast of the bronchial tree for experimental purposes was produced by West and Hugh-Jones in 1959. These workers took a solid resin cast produced by Tompsett and trimmed all the bronchi distal to the first branching of the segmental bronchi. A flexible hollow cast of the solid model was then produced and a further solid cast made in dental wax. This wax tree was embedded in a block of clear resin with extensions leading from the segmental bronchi to the outside. The wax was melted out leaving a hollow replica of the upper bronchial tree. Patterns of flow were studied by observing the flow of dye in water and different gases through the cast.

Dekker (1961) produced a similar type of cast of the trachea, with and without the larynx. He used these casts to investigate the effect of the larynx on the onset of turbulence.

A severe limitation on the usefulness of these embedded casts is the small number of airways through which flow can be produced. Access to the innermost airways is blocked by those at the periphery. We (1970) overcame this difficulty by making a hollow cast in which the walls were very thin (0.20 in.) and not enclosed in a block of plastic. This was produced by first making a solid wax cast of the airways when the lung was inflated under negative pressure in an artificial thorax. The tissue was digested off the solid cast in a bath of concentrated hydrochloric acid. A coating of colloidal silver was sprayed over the solid cast to provide a conducting layer and the whole cast silver plated until the walls were of the required thickness. To enable a stream of gas to be passed through the cast the ends of the small bronchi should be open. To ensure this was so, a blob of wax was placed over the colloidal silver at the ends of the bronchi. This prevented electro-plating at these points and when the wax was melted out of the cast these airways were left open.

At about the same time, Eisman produced a hollow lung cast by coating a solid metal cast with latex and then melting out the low melting point metal.

Hollow lung casts provide an attractive alternative to real lungs for placing of instruments such as a hot wire animometer. Such measurements are being made by Schroter and Sudlow to investigate the velocity profiles across airways of the human lung.

Hollow casts represent a model of the lung 'frozen' at some point in the respiratory cycle. They provide an opportunity to investigate the effect of bronchial tree anatomy on respiratory dynamics without the interfering effect of lung compliance and a gradient of pleural pressure. We have used hollow casts to investigate the deposition of fibres in the lung and to estimate the site of airways resistance. Transit times for a gas front to pass from the carina to small airways has also been measured. In all these investigations the similarity between hollow casts and excised lungs demonstrates the influence of structure on function in the bronchial tree.

The nature of the air spaces within the mammalian lung is of primary importance to its function in health and disease. Over the past three hundred years the understanding of this function has been greatly advanced by the use of solid casts. It is probable that at least as much information can now be gained by the application of experimental techniques to hollow casts, produced by methods outlined five hundred years ago by Leonardo da Vinci.

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ANDREW DAVIES

LEAD POISONING IN THE ANCIENT WORLD

Lead was one of the seven metals of antiquity. Its discovery dates back to at least 3500 B.C. and lead artefacts have been discovered widespread throughout the ancient world.

Lead does not occur in an elemental state in nature although its sulphide ore galena (PbS) is common. It is probable that galena was first used in antiquity for making into ornaments or for use as an eye paint.[1] The discovery of metallic lead may well have

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GAS TRANSIT TIMES IN THE PIG LUNG

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(*Received 17 January 1975*)

SUMMARY

1. A method of measuring the time taken by a front of gas to pass from the carina to individual small airways in the lung is described.

2. This method has been used to investigate the movement of a gas front through hollow lung casts, excised lungs, and excised lungs subjected to a gradient of pleural pressure.

3. The results obtained support the suggestion of morphometric studies that the asymmetrical anatomy of the bronchial tree plays a part in the uneven distribution of ventilation in the lung.

4. The effect of a gradient of pleural pressure over the surface of the upright lung was to increase the time taken by a gas front to reach dependent regions.

INTRODUCTION

Two main types of inequality of ventilation exist in the mammalian lung. The first, stratified inhomogeneity, results from the fact that as a front of gas passes down the bronchial tree its forward velocity decreases due to the increasing cross-sectional area of the airways. The reduction of velocity continues until in the fine airways diffusion dominates bulk flow in determining the forward movement of gas molecules. This results in a gradient of concentration along the airways.

The second type of inequality, regional inhomogeneity, is the result of unequal distribution of ventilation among the terminal units of the lung. This inequality may exist in terms of time (sequential ventilation) where regions filling early in inspiration receive a larger proportion of dead space gas than those filling later; or in terms of space, where inspired gas has to traverse different distances from the lips to the respiratory surfaces. This implies that different amounts of dead space gas will precede inspired air into different respiratory regions of the lung.

Measurements of airways resistance at different levels of the bronchial tree (Macklem & Mead, 1967) suggest that the resistance to flow offered

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by the airways has only a small influence on the distribution of gas in the lungs, and that bulk flow is largely governed by the elastic distensibility of the air spaces and the forces distending them.

Unequal distribution of dead space gas implies that the time taken by a front of gas to reach the respiratory region varies throughout the lung. Ross (1957) defined this interval as 'transit time'. Cumming (1967) has pointed out that if diffusion is complete the degree of uneven ventilation in the lung will be determined by those factors affecting this transit time. In the present study transit time is the time taken by a front of gas to pass from the carina to any defined point in the bronchial tree.

Direct measurements of transit time from the carina to points within the pig lung have been made, and the effect of the asymmetrical anatomy of the bronchial tree, the compliance of the lung, and the presence of a gradient of pressure over its surface have been investigated to determine their effect on equality of ventilation in terms of transit time.

METHODS

To measure transit time from the carina to individual small airways in the lung a gas front was 'labelled' with a halogenated hydrocarbon, and its arrival detected at a point after injection into excised lungs (weight 200–370 g), or hollow casts of the bronchial tree. The casts were silver and reproduced the branching of the airways in great detail (Timbrell, Bevan, Davies & Munday 1970). They were produced by electroplating a layer of silver on to a solid wax cast of the bronchial tree and then melting out the wax. To produce the solid casts lungs were inflated by the method of Weibel (1963) to approximately three quarters total lung capacity, and paraffin wax at 80° C poured into the main bronchus. The absence of any prior fixing or drying, and the use of a low density material (wax) reduced the risk of shrinkage and distortion of the tissues to less than that found in conventional techniques. The wax was made radio-opaque by the addition of iodized poppy seed oil (Lipiadol). The wax filled lung was X-rayed in three aspects to ensure penetration of the airways had been complete. After digesting the tissues in concentrated hydrochloric acid and washing the solid wax cast it was sprayed with a conducting layer of colloidal silver to enable electroplating to take place. To prevent plating at the ends of the airways, and so ensure they remained open, a loop of fine wire carrying a film of molten wax was passed over the end of the airway. This deposited an insulating sheath of wax over the colloidal silver. With practice the distance the loop was taken along an airway, and hence the final diameter of the airways of the hollow cast could be quite well controlled. The airways were terminated at about 0·1 cm diameter. The peripheral ends of the airways were open to allow air flow through the casts. These hollow casts provided a model of the bronchial tree from which samples could be drawn at accurately known distances from the carina.

The excised lungs and hollow casts were ventilated by a positive pressure pump at a frequency of 12 cycles per minute and a stroke volume of 250 ml. The output of the pump approximated to the positive half of a sine wave. The isolated lungs were allowed to deflate under their own elastic recoil during the expiratory phase and the pressure in the ventilating system reached atmospheric well before the next cycle commenced. The ventilating pressure at the end of inflation in all lungs was about

20 cm water. This and the fact that all the lobes of a lung filled uniformly and synchronously suggests that large differences in compliance did not exist between lobes. While being ventilated the lungs were placed in a saline filled tray and covered with a cloth moistened with saline.

At a predetermined point at the beginning of the ventilation cycle a 1 ml. slug of dichlorodifluromethane gas was rapidly injected into the centre of the main airway, at a point above the carina, by a solenoid operated syringe.

The arrival of the halogenated gas front at a point within the lung or cast was detected by a commercial halogen sensitive diode (Genevac L.D. 1) having a response time of 75 msec for 90% full response. This instrument is reported to have the sensitivity of a helium mass spectrometer, but is much more resistant to contamination (Torney, 1957).

A vacuum pump drew a small continuous sample of gas (< 5 ml./min) through the diode via a catheter implanted at the chosen sampling site. The pump was of sufficient capacity to maintain the pressure at the diode below 0·5 torr which was necessary for its efficient operation. A rapidly responding ultra violet recorder (Southern Electronics 3006) displayed the output of the diode on a moving trace.

A second diode, sampling gas at the level of the carina, acted as a fixed reference point. The time interval between the signals from the two diodes (after allowing for the delays due to the sampling catheters) represented the transit time between the two sampling sites. All records were read to the nearest 50 msec.

In hollow casts the catheters sampled from a series of 1 mm diam. holes drilled in the cast wall. In excised lungs a retrograde catheter was used. This consisted of a polyethylene tube having a collar at one end of the same diameter as the airway from which the sample is required. The catheter was positioned by the method of Macklem & Mead (1967), with the collar wedged in an airway and the tube extending peripherally through the parenchyma and pleura. The free end was connected to the halogen sensitive diode. By using several catheters it was possible to detect the arrival of a labelled gas front in airways of the same diameter situated in different regions of the lung.

The experiment is shown diagrammatically in Fig. 1. To study the effect of a gravity induced gradient of pleural pressure on transit times in excised lungs a fluidized bed was used (Schroter & Sudlow, 1969). This consisted of a deep bed of solid particles (slightly expanded polystyrene spheres approximately 1 mm diameter) through which was passed a uniform up-current of air. Under these conditions the mass of the bed behaved like a low density liquid. The bulk density of the bed depended on the degree of expansion of the polystyrene. At the expansion used in these experiments the pressure measured with a thin latex balloon connected to a water manometer increased by 0·25 cm H_2O/cm below the surface of the bed. The volume of the fluidized spheres used was just sufficient to cover the apex of the inflated lung. The lungs were supported in the bed, by threads attached by pinch clips to their margins and hilum, in positions corresponding to the upright, supine, and inverted positions in man. Katsura, Rosenewajg & Sutherland (1970) have demonstrated that this type of support does not affect distribution of ventilation in the lung. The rigid tube used to ventilate the lung helped to locate it in the fluidized bed and prevent undue strain on the points of support at its margins. The lungs were ventilated at a frequency of 12 c/min and a stroke volume of 250 ml. Pairs of retrograde catheters of the required diameter were embedded by the method described, one in the upper lobe, the other in the lower. Transit times were measured in the upright, supine, and inverted positions in the fluidized bed.

After transit time measurements had been concluded on a lung the airways in which the catheters had impacted were dissected out and their distance from the carina measured with dividers. Bronchiographic evidence (Macklem, Frazer &

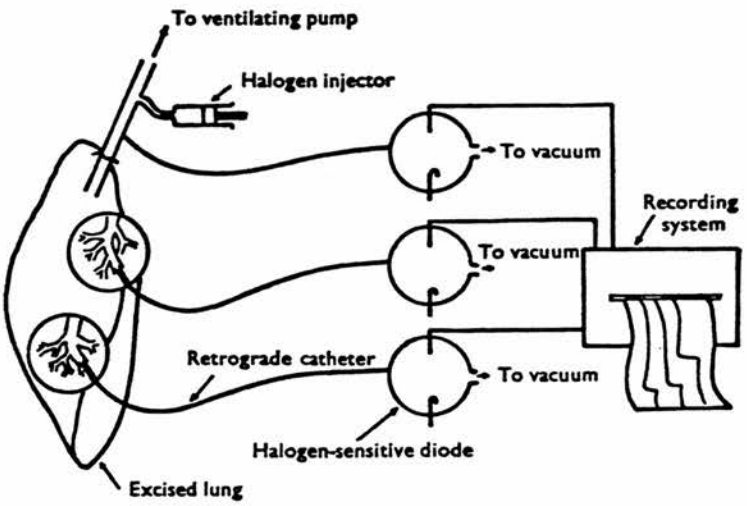

Fig. 1. Diagram of the experiment.

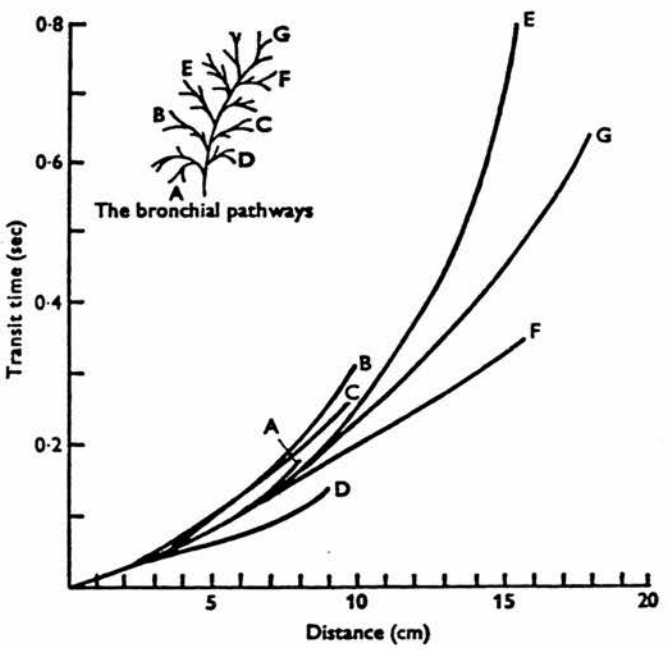

Fig. 2. Advance of gas front through seven bronchial pathways of a hollow cast. Transit times were measured at intervals of 1 cm along the seven pathways (shown inset).

Bates, 1963) and studies on excised tissue (Martin & Procter, 1958) demonstrate that bronchial length is little altered through the respiratory cycle. Extrapolating to the deflated condition I suggest that this measurement is a good estimate of the distance from the carina in the inflated lung. Dissection of the region in which a catheter had impacted did not reveal any distension of the airway, indicating that the diameter of a catheter collar was a good estimate of the diameter of the airway in which it would impact.

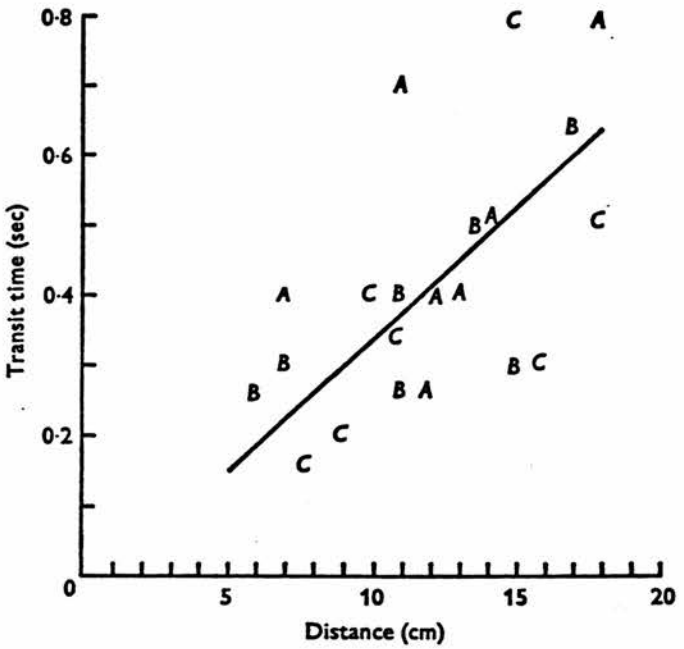

Fig. 3. Plot of transit time against distance from the carina for terminal sample points in three casts *A*, *B* and *C*.

RESULTS

When transit times from the carina to points in a hollow cast were measured, the results shown in Fig. 2 were obtained. The measurements were made at 1 cm intervals in seven bronchial pathways. The relationship between transit times through the various branches of the cast remained essentially the same whether the cast was ventilated in a cyclic fashion or by a steady flow of air (4 l./min) down the main bronchus.

The process of drilling 1 mm diameter sampling holes through the wall of the cast was continued peripherally until the airway became too narrow to withstand the procedure. At that point the airway was about 0·15 cm diameter. The relationship (correlation coefficient 0·7) between transit time to these terminal sample points and their distance from the carina is

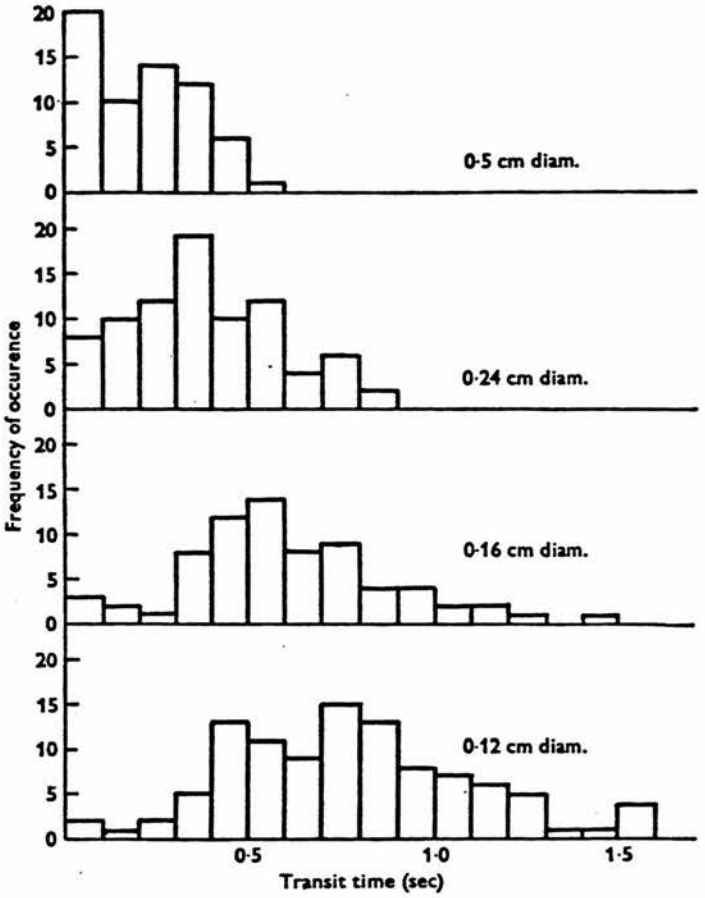

Fig. 4. Distribution of transit times to airways of four diameters in excised lungs.

shown in Fig. 3. This represents results obtained with three casts. Analysis of variance showed no significant difference existed between casts.

The results of measuring transit time to airways of 0·5, 0·24, 0·16 and 0·12 cm diameter in thirty-five excised lungs are shown in Fig. 4. Transit times have been grouped into intervals of 100 msec. An analysis of variance showed no significant difference between lungs, the distribution of transit times arising from factors within individual lungs.

If transit times to airways of a particular diameter are plotted against their distance from the carina, regression lines of different slopes result (Fig. 5). The arithmetic mean for each airway size is shown and the regression lines continued 1 S.D. (in terms of distance) either side of the mean.

A number of lungs were subjected to a vertical gradient of pleural pressure, and transit time measured to airways of 0·5, 0·24, 0·16 and 0·12 cm diam. with the lungs consecutively in the equivalent of the supine, head up, supine, head down and supine positions. Whether the catheter lodged in the upper or lower lobe of the lung was noted. The average transit times to 0·12 cm diameter airways with the lung in the various positions is shown in Fig. 6. Transit times to other diameter airways were affected by position in the pressure gradient in the same way.

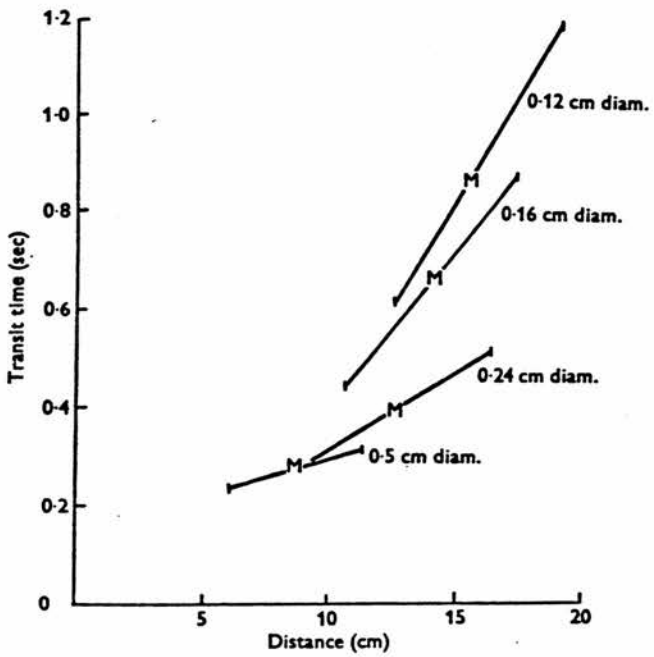

Fig. 5. Transit time plotted against distance from the carina in airways of four different diameters in excised lungs (mean and 1 S.D. in terms of distance shown).

DISCUSSION

The effect of bronchial tree morphology on the distribution of ventilation has been discussed by Ross (1957) and Horsfield & Cumming (1968). They suggested that the basic form of unequal ventilation results from asymmetrical anatomy, and on this, inequality from other causes is superimposed. They expressed their findings in terms of a distribution of transit times. The present study was designed to investigate if such a distribution could be measured in the lung, and if so the effect on it of factors known to influence the uniformity of ventilation.

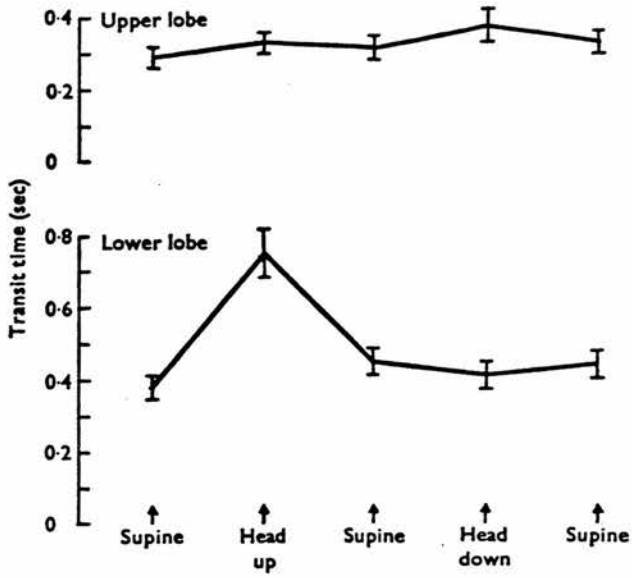

Fig. 6. Mean transit time to 0·12 cm diam. airways in lungs suspended in three positions in a vertical gradient of pleural pressure. (Position affected transit time to other diameter airways in the same manner.)

The possibility that the shape of the halogenated gas front used in this study was different at the different sample points, and that this may have affected the measurement of transit time needs to be considered. At the level of the peripheral sampling sites there would have been considerable secondary motion within the gas stream (Schroter & Sudlow, 1969) causing mixing across the airway. In addition, diffusion is so rapid that in a 1 mm diameter airway radial mixing is forty times faster than forward flow (Crank, 1957) obliterating radial differences in concentration and resulting in a flat front. However a concentration gradient may have extended along the airways. The detectors used in this study were triggered when the concentration of halogen exceeded a certain very low value. It was the detection of this concentration which was taken as the arrival of the gas front at the sampling site.

Many studies of the aerodynamic properties of mammalian airways have been made in cats, dogs and monkeys. Since it is generally accepted that the branching of the lung airways is adapted to the shape of the thoracic cage great care should be exercised in drawing parallels between the lungs of different species. The pig was chosen for the present study on grounds of size and anatomy, in the hope that the data obtained would in some degree be applicable in both man and laboratory animals.

Wagner, Latham, Brinkman & Filley (1969) measured the time taken by carbon monoxide injected at the larynx to appear in the pulmonary capillary blood of dogs. Their observations were restricted to a single area of the lung's surface and consequently they could not measure the distribution of transit times that existed within the lung. This difficulty was overcome in the present study by detecting the arrival of a gas front at multiple points within the bronchial tree.

Transit time through any segment of the bronchial tree is related to the length and cross-sectional area of that segment. In both casts and lungs transit times of a gas front passing from the largest to the smallest airways were related to distance from the carina in a curvilinear fashion (Figs. 2 and 5). The curvature of the line reflects the increase in cross-sectional area of the airways. When transit times were measured to airways of a single size only a small portion of this curve was represented. In these cases the relationship between transit time and distance could be represented by a straight line (Figs. 3 and 5).

For the hollow casts to reflect the behaviour of whole lungs the down stream resistance of all the open ended bronchi should be the same. This will be the case as they were all pruned to the same diameter and discharge into free air. They can be considered to subtend infinitely compliant air spaces under uniform pleural pressure.

The casts represent a model of the lungs in which regional changes in volume are abolished, in which case the only determinant of distribution of ventilation is the structure of the bronchial tree. In this way the effects of the morphology of the bronchial tree on transit time can be assessed in isolation, and compared with compliance and the gradient of pleural pressure as a factor affecting distribution of ventilation.

The results obtained in the present study with hollow casts are an experimental extension of the morphometric investigations of Ross (1957) and Horsfield & Cumming (1968). They support the suggestion of these workers that the asymmetrical anatomy of the bronchial tree results in a distribution of transit times, and hence uneven ventilation, throughout the lung.

In both hollow casts and excised lungs transit times to airways of a particular diameter were related to their distance from the carina. The functional consequences of this are (1) dead space gas is unevenly distributed in the lung, (2) the gaseous interface between inhaled and dead space gas is established at different distances from the respiratory surface, and (3) the time which an interface spends in the respiratory region varies throughout the lung. An important influence on these factors is the increasing cross-sectional area of the bronchial tree. This results in a rapid reduction in the linear velocity of the advancing front as it reaches the

peripheral airways (Fig. 5) which means that for a large part of the respiratory cycle the gas front is almost stationary in the respiratory region of the lung, a fact indicated by Johnson & van Liew (1974) in their demonstration of the importance of molecular diffusion in oxygen transport to the alveolar wall. The slowing of an advancing gas front is seen in hollow casts ventilated by a constant flow. It appears therefore to be the result of increasing cross-section rather than an effect of compliance or cyclic ventilation.

The similarity between the regression of transit times to 0·16 cm diameter airways against their distance from the carina in casts and excised lungs indicates that any lobar differences in compliance were not great enough to affect the relationship between transit time and distance that resulted from bronchial anatomy.

The effect of gravity on the lung must be considered in any measurement of transit time. It has repeatedly been shown in studies of bronchiospirometry (Koler, Young & Martin, 1959) by scanning the lungs after inhalation of ^{133}Xe (Dollfuss, Milic-Emili & Bates, 1967) that the distribution of ventilation at low flow rates is mainly related to regional expansion of the lung.

Bake, Bjure, Grimby, Milic-Emili & Nilson (1967) suggested that lobar differences in lung compliance exist in man similar to those found in dogs (Faridy, Kid & Milic-Emili, 1967) and that these interact with the gravity induced gradient of pleural pressure. The fact that such a gradient affects the distribution of ventilation has been demonstrated *in vivo* (Ball, Stewart, Newsham & Bates, 1962; Bryan, Bentivoglio, Beerel, MacLeish, Zidulka & Bates, 1964; Milic-Emili, Henderson, Dolovich, Trop & Kaneko, 1966) and in excised lungs (Zardini & West, 1966).

In lungs subjected to a gradient of pleural pressure increasing from apex to base, transit times increased to dependent regions when the lung was in the 'head up' position. In the 'head down' position transit times to what was normally the lower lobe were not significantly different from the values in the supine position. This was probably due to the sampling sites in the lower lobe being about the same distance below the surface of the fluidized bed in both the supine and inverted position. The relative immunity of transit times in the upper lobe to changes in the pleural pressure gradient was ascribed to the shorter vertical distances from the sample sites in this area to the carina.

The increase in transit time to dependent regions can be explained in terms of effective compliance. A region did not expand until its internal pressure exceeded the pressure due to the fluidized bed. This parallels the behaviour of the lung in an intact animal, where regions exposed to the most negative pressure expand first at the beginning of inspiration,

causing air flow to the upper regions. Horsfield (1967) has demonstrated that the mean airway path length in man is slightly greater to the lower than to the upper lobe. The effect of gravity on the upright lung will therefore be to increase the distribution of transit times that would arise from purely anatomical reasons.

The results obtained in the present study suggest that the anatomy of the bronchial tree influences distribution of ventilation in the lungs as predicted by morphometric studies. This has been demonstrated as a distribution of transit times and as a reduction in the velocity of a gas front passing into the lung. The similarity between the advance of a gas front into a rigid cast and into an excised lung suggests that the differences in compliance that exist in such a preparation are not large enough to significantly affect the distribution of transit times. A gradient of pleural pressure over the lung however produces a significant increase in transit times to the lower lobe when the lung is in the upright position. The effect of the naturally occurring gradient of pleural pressure in upright man would therefore be to accentuate the effect of bronchial tree architecture on the distribution of ventilation.

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Publication 7.

Davies,A & Widdicombe,J.G. (1975)

Effects of chronic sulphur dioxide exposure on lung reflexes.

Proc. International Conference of Pathophysiology (Prague).

EFFECTS OF CHRONIC SULPHUR DIOXIDE EXPOSURE ON LUNG REFLEXES

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As part of a study of an animal model of bronchitis (As described by Widdicombe, this meeting) rabbits were exposed to 150ppm sulphur dioxide for twelve three hour periods. One week after the last exposure the rabbits were anaesthetised and lung mechanics, pattern of breathing and lung reflexes were recorded. During and after exposure frequency of breathing was significantly slowed. There was no significant change in total pulmonary resistance. The strength of the Breuer-Hering inflation reflex was significantly reduced. The ratio of stretch receptors to rapidly adapting receptors was 8:1 in the control animals compared to 10:1 in controls. The significance of these changes will be discussed.

Publication 8.

Widdicombe,J.G. Davies,A. & Dixon,M.(1975)

Modelling of asthma and bronchitis in animals.

In: Progress in Pathophysiology, pp316-318.
Czechoslovak Academy of Sciences, Prague.

MODELLING OF ASTHMA AND BRONCHITIS IN ANIMALS

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A disease can be defined in several ways: by its clinical characteristics, by the underlying pathological changes, or by the disturbances of function which are associated with it. Without an adequate definition of the disease, the relevance of any animal model is bound to be tenous.

Asthma is a collection of diseases which have in common the partial obstruction of the bronchial tree due to smooth muscle contraction. There is great variability in other features — mucosal swelling, mucus secretion, dyspnoea, airway hyperresponsiveness and the involvement of immunological processes. Two types of asthma are usually distinguished: „extrinsic asthma", characterized by bronchoconstrictor responses to inhaled aerosols, irritant gases and antigenic substances, and „intrinsic asthma" which lacks these features but is more continuous and refractory to treatment. The exact role of immunological processes has been defined for neither type of asthma (Bouhyus, 1974; Porter and Birch, 1971).

The classical way to mimic asthma in experimental animals is to sensitize them to a foreing protein and then at a later date to challenge them with small doses of inhaled or injected antigens. Guinea-pigs and rabbits have been chiefly used, with egg or serum albumen as the antigen. The model resembles human asthma in that the challenge causes a reversible bronchoconstriction due to smooth muscle contraction. It differs from asthma in that a different immunoglobulin fraction is involved, IgG rather than IgE, and that the chemical mediators of the bronchoconstriction may differ from those in man. If the challenge is by intravenous injection of the antigen then severe systemic reactions may complicate the picture.

A model more relevant to clinical asthma is the allergy to parasitic worms or pollen seen in dogs (Gold et al., 1972). When challenged by an inhalation of antigen, the dogs respond with a prompt bronchoconstriction which seems to resemble asthma in most respects, and is blocked by atropine. The antigen

setting up this reflex bronchoconstriction is effective when its penetration is limited to the airway epithelium, where the efferent endings for reflex bronchoconstriction are located (Richardson et al., 1973).

These models of asthma have led to the clarification of several processes in allergic bronchoconstriction. A large number of chemical mediators may be released in the lungs, which can have direct actions on the smooth muscle there or on nervous recptors. Afferent endings (irritant receptors) in the airway epithelium are stimulated, causing reflex bronchoconstriction, hyperpnoea and probably breathlessness (Widdicombe, 1971). There is accumulating evidence that similar mechanisms are involved in human extrinsic asthma. Thus the bronchoconstriction is often sensitive to atropinic drugs (Boehringer Symposium. 1975), and is associated with hyperventilation and breathlessness, all of which suggest a reflex component.

Experimental bronchitis is easy to study, but the results are more difficult to interpret than for asthma. This is because there is no statisfactory definition of chronic bronchitis. Clinically it is „the condition of subjects with chronic or recurrent excessive mucus secretion in the bronchial tree". This clinical definition is highly unsatisfactory from the pathophysiological point of view, and there is no adequate method of measuring the output of respiratory tract mucus in patients. Pathologically there is mucus gland hyperplasia in chronic bronchitis, but this is nearly always associated with variable degrees of chronic inflammatory and emphysematous changes. Physiologically there may be a mixture of expiratory airflow obstruction, increased lung volume, mixing defect and blood gas changes, but none of these is definitive.

Since chronic bronchitis cannot be defined by scientific criteria, it is impossible to set up a convincing model of the disease. The problem is by-passed by exposing animals to the same environmental conditions which are known to cause chronic bronchitis in man, namely inhaled irritant gases or smokes. It is then assumed that the resulting lung condition is analogous to chronic bronchitis, although one cannot apply the definitive criterion — increased mucus production.

With this kind of model treated animals show goblet cell and mucus gland hyperplasia, but other physiological changes have not been adequately assessed. We have studied changes in reflex activity from the lungs (Davies, this meeting), but it is not possible to say how closely the animal model corresponds to the human condition. Much additional work is needed, together with a clearer understanding of human bronchitis.

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Publication 9.

Davies,A.(1976)

Respiratory reflexes in rabbits without pulmonary stretch receptor activity.

In INSERM Symp. on Neural Control of Respiration, Duron,B.Ed. vol59,pp,253-262..

Les Colloques de l'Institut National de la Santé et de la Recherche Médicale
Respiratory centres and afferent systems
Centres respiratoires et systèmes afférents
Duron B. Editor. INSERM. 4-6 March 1975. vol. 59. pp. 253-262

Respiratory reflexes in rabbits without pulmonary stretch receptor activity

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Exposure for 10 min to an atmosphere of 200 p.p.m. sulphur dioxide in air selectively inhibits pulmonary stretch receptor activity in rabbits (1). We have tested a series of lung reflexes elicited before and after stretch receptor inhibition, in the hope of separating the part played by the various lung receptor systems in respiratory reflex behaviour, and in determining the pattern of normal quiet breathing. The experiments were carried out on groups of rabbits, each group subjected to some or all of the procedures to be outlined.

The animals were New Zealand White rabbits weighing from 2.5 to 3.5 kg. They were anaesthetized with sodium pentobarbitone (30-40 mg/kg) and femoral arterial and venous catheters were inserted. The pattern of breathing and lung compliance and total lung resistance were recorded by conventional methods, using tracheal and intrapleural cannulae. Both cervical vagus nerves were exposed, and when receptor activity was studied this was recorded from single fibres in the cut right vagus nerve.

Phrenic activity was recorded from multifibre strands of the uppermost root of the right phrenic nerve. The impulses were rectified and integrated against time. The integrated record was zeroed automatically at the end of inspiration. The record from the integrator was S-shaped, and varied slightly from animal to animal.

We assessed the integral by calling the peak amplitude of the signal "integrated phrenic height", and the angle between the horizontal and a line joining the beginning and end of phrenic activity we have called "phrenic slope".

Sulphur dioxide mixtures were prepared by mixing the pure gas with air in a Douglas Bag. They were administered by allowing the animals to inhale the mixture from a passing stream of the gas. After 10 min or less of this treatment changes in the pattern of breathing were observed which were intermediate between the control and the vagotomised states (Fig. 1).

After inhalation of SO_2, tidal volume (V_T) and inspiratory duration (t_I) were increased, as was also seen after vagotomy. However pulmonary stretch receptor block differed from vagotomy in its effects on expiratory duration

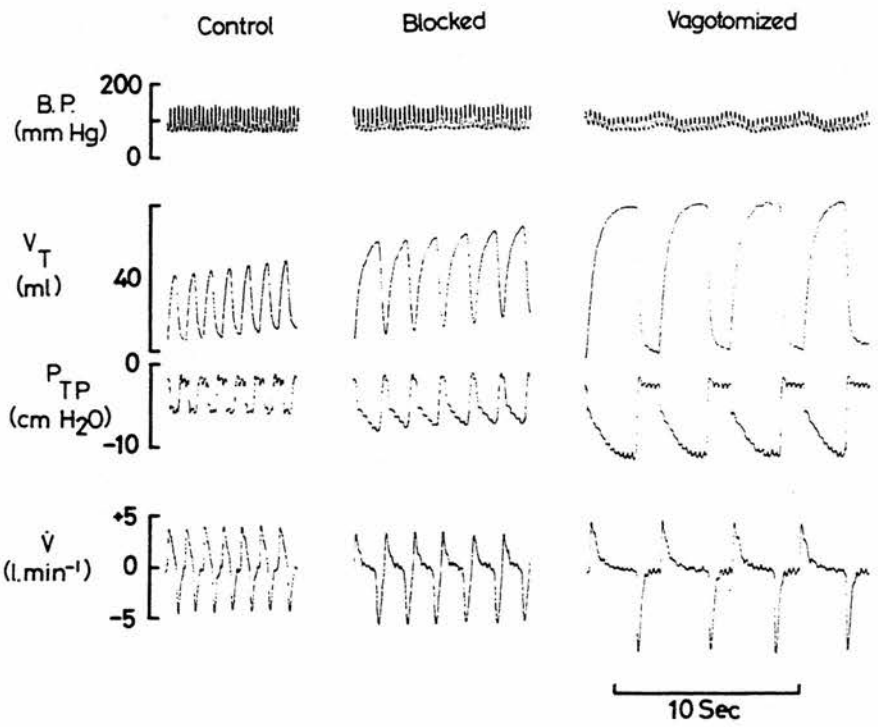

Fig. 1. Effect of pulmonary stretch receptor block by SO_2 (middle panels), compared with control (left) and vagotomy (right) on, from above downwards: blood pressure, tidal volume (with some integrator drift), transpulmonary pressure and airflow. For further description see text.

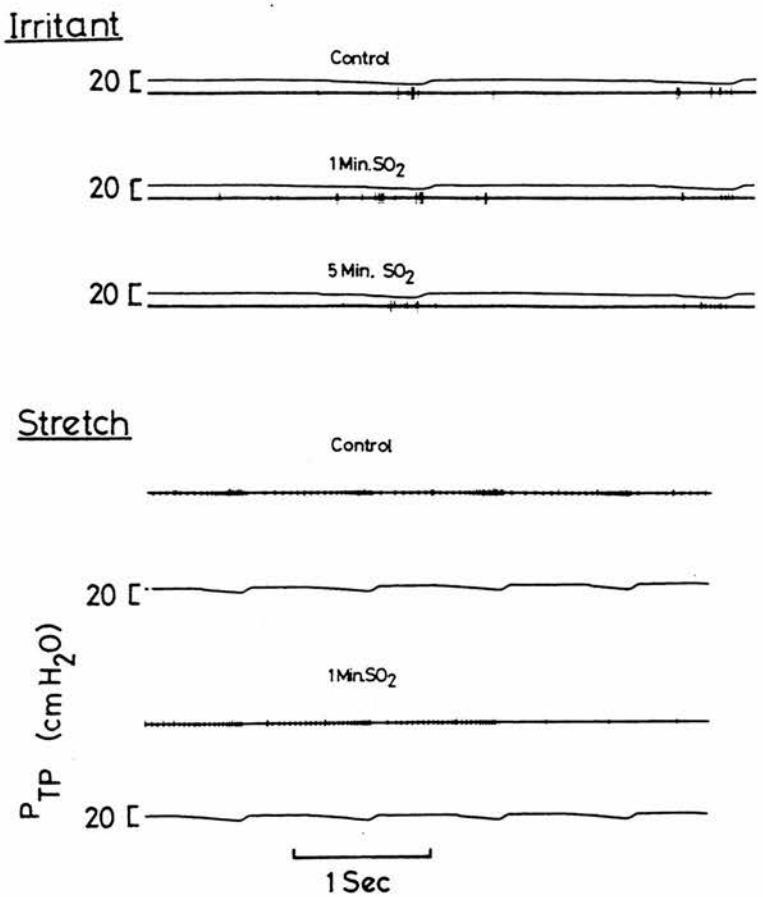

Fig. 2. Effects of 200 p.p.m. SO_2 on (above) irritant receptor and (below) pulmonary stretch receptor discharge. Traces are single fibre activity in the vagus nerve and transpulmonary pressure. Note that the pulmonary stretch receptor is paralysed after 1 min, but the irritant receptor is unaffected after 5 min of SO_2.

(t_E); in the blocked state t_E was shortened while in vagotomy it was increased.

The reasons for these changes in the pattern of breathing due to SO_2 can be seen from records of activity in fibres from lung stretch and irritant receptors while SO_2 was being administered (Fig. 2).

The irritant receptor recorded in Fig. 2 would tolerate 200 p.p.m. SO_2 almost indefinitely, while the stretch receptor was rapidly inactivated by this treatment. Irritant receptor activity was never abolished by SO_2 whereas nearly all pulmonary stretch receptors were completely paralysed by SO_2 (1).

One of the tests we used as an index of pulmonary stretch receptor activity was the strength of the Breuer-Hering inflation reflex, measured as the ratio of the pause produced by positive pressure inflation of the lungs with 10 cm H_2O to the length of the pre-inflation breath. Inhibition of stretch receptors by inhalation of SO_2 reduced the pause ratio to approximately one, i.e. there was no inhibition of breathing and the Breuer-Hering reflex was abolished.

We studied the effect of an increase in the inspired concentration of CO_2, which increased V_T, minute volume and the frequency of breathing. The increase in frequency and ventilation were greater in the pulmonary stretch receptor-blocked animals than in the control of vagotomised rabbits. Similarly, with V_T inhibition of stretch receptors produced an enhanced response to CO_2 compared with the controls. The combination of these two effects resulted in a steepening of the end-tidal CO_2/ventilation curve to a value greater than for the controls or the rabbits after vagotomy (Fig. 3).

When breathing was accelerated by CO_2 in normal conditions, after pulmonary stretch receptor block and after vagotomy, there was always a significant increase in the slope of the phrenic integral. This was in contrast to several other states of accelerated breathing.

Histamine was injected intravenously into the rabbits before and after stretch receptor block. This injection resulted in small insignificant changes in t_I accompanied by larger reductions in t_E which were unaffected by abolition of stretch receptor activity. Whether stretch receptors were active or not and whether breathing was driven by histamine or not, the phrenic integral had the same rate of increase (slope) until termination of inspiration put an end to phrenic activity.

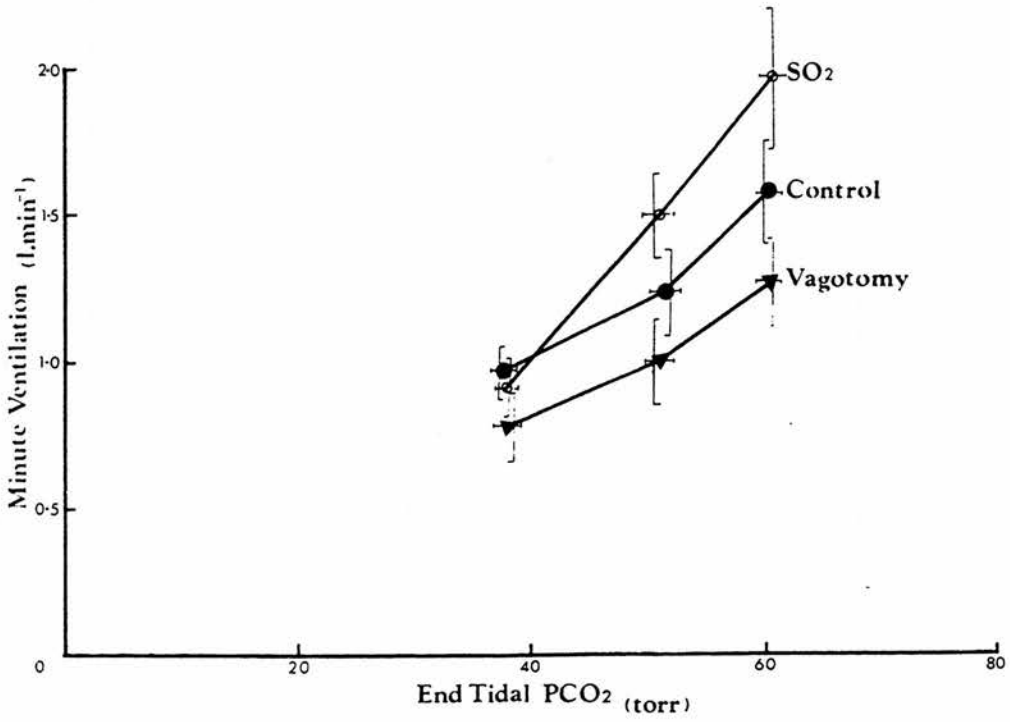

Fig. 3. CO_2/ventilation relationships for control rabbits, those with pulmonary stretch receptors blocked by SO_2 and those with vagotomies. For further description see text.

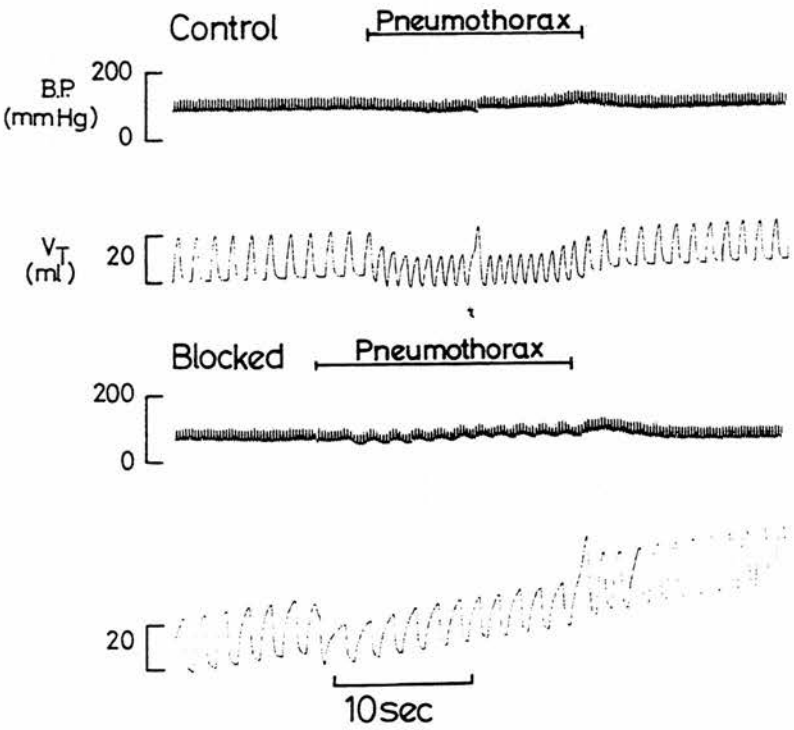

Fig. 4. Effect of pneumothorax (during signal bars) on blood pressure (above) and tidal volume (below, with some integrator drift). Control, upper record; during SO_2 block of pulmonary stretch receptors, below. Note prolongation of t_I on induction of pneumothorax with stretch receptor block.

Similarly, with injections of phenyl diguanide, the slope of the phrenic integral was not altered during the subsequent breathing in any condition of pulmonary stretch receptors or vagal conduction.

Some of the methods we have used to elicit reflexes may be open to the criticisms that there may have been some pulmonary stretch receptor activity present, and that more than one group of lung receptors may have been stimulated by procedures designed specifically to act on a single reflex system. On the induction of pneumothorax, however, stretch receptor activity is suppressed by the same action which stimulates lung irritant receptors, and J-receptors are not appreciably affected. When a pneumothorax was induced in an animal with intact stretch receptor activity there was a small increase in the t_I of the first breath, and a large decrease in t_E and V_T. In the animal with stretch receptor activity abolished the response was altered. The increase in t_I in the first breath was significantly greater, and the reduction in t_E significantly less than in the control conditions. The duration of phrenic discharge in all animals reflected changes in t_I, but there were no significant changes in phrenic slope(Fig. 4).

Finally we can compare the effects of abolishing stretch receptor activity with the effects of vagotomy, of which the former can be considered an "intermediate" stage. In both cases t_I was increased, although with stretch receptor block this increase in t_I was accompanied by a reduction in t_E. V_T was increased in both cases. The phrenic integral slope was not significantly altered by either procedure.

Discussion

We have seen from single fibre recordings that we can abolish the activity of pulmonary stretch receptors in rabbits by administration of SO_2 (1), and with this effect come changes in the pattern of breathing and reflex behaviour, some of which can be easily explained and some of which are more difficult to account for.

The effects of pulmonary stretch receptor block on the decreases in t_I produced by injected histamine or phenyl diguanide can be explained in terms of sensitization of stretch receptors by these drugs, either by direct action on the surrounding lung tissue in the case of histamine (2), or by the increases in functional residual capacity which always accompanied the ventilatory effects of phenyl diguanide. In the case of our observations on the first breath of pneumothorax, we can say that pulmonary stretch receptors are suppressed (3,4) and J receptors may be transiently and weakly stimulated, and then only by extreme degrees of lung collapse (5). This leaves irritant receptors as the

most likely cause of the increase in t_I. Supporting this suggestion is the fact that both irritant receptor activity and the lengthening of t_I diminished as pneumothorax proceded (6).

The loss of the Breuer-Hering inflation reflex we have described ties in precisely with the inactivation of the volume sensing receptors described by Adrian (7).

The changes in t_I we have seen with SO_2 inhalation have been observed in other differential blocks of vagal transmission such as by cold (8,9) or by anodal block (10), and in local anaesthesia of the airways (11). Vagotomy further increases t_I which suggests that a shortening mechanism still exists after stretch receptor block. This might be the few stretch receptors left intact after inhalation of the SO_2, or the activity of irritant receptors which would be stimulated by the increase in V_T produced by the stretch receptor block. That the two variables V_T and t_I are linked has been demonstrated by Clark and von Euler (12). Stretch receptors have the role of volume sensors, switching off inspiration at the appropriate volume. The time taken for this volume to be reached will of course depend on the rate of inflation of the lungs and this in turn depends on phrenic activity. Increased inspired CO_2 concentrations in our experiments increased phrenic slope, and therefore reduced the time taken for volume to reach the critical value. The increase in V_T when stretch receptors are blocked by SO_2 is seen in other types of differential vagal block (loc.cit). The further increase on vagotomy suggests a residual stretch receptor activity or enhanced irritant or J-receptor activity which has a limiting effect on V_T and t_I in the blocked state.

Unlike the changes in V_T and t_I, the changes in t_E produced by SO_2 were not in the same direction as those produced by vagotomy. SO_2 block reduced t_E while vagotomy lengthened it. The decrease in t_E with SO_2 can be explained in terms of a removal of the tonic stretch receptor activity which tends to prolong t_E. The removal of all vagal activity by vagotomy and the subsequent lengthening of t_E suggests that the activity of irritant and possibly J-receptors has a t_E terminating effect. Support for this suggestion comes from the work of Nadel et al (13) who blocked the lung stretch reflex in dogs by vagal cooling but left the 'irritant reflex' to histamine aerosols intact. This condition also caused a reduction in t_E.

Our evidence then seems to support the suggestion that the characteristics of individual breaths are determined centrally by a mechanism with a primary intrinsic rhythm.

This basic plan can be altered however by changes in pulmonary stretch and irritant receptor activity. Increases in activity of these two groups of receptors have t_I-terminating and t_I-extending roles respectively by altering the duration of phrenic discharge but not its rate of increase. t_E on the other hand is increased by stretch and shortened by irritant receptor activity.

Our experiments on animals without stretch receptor activity suggest that the patterns of breathing seen in the reflex conditions we have studied consist of breaths which are smaller or larger parts of breaths originally designed centrally.

SUMMARY

We have studied the actions of lung reflexes in rabbits with pulmonary stretch receptor activity depressed by inhalation of 200 p.p.m. SO_2, and compared the responses with those in control animals and those vagotomized. Pulmonary stretch receptor blockade enhanced the ventilatory response of the rabbits to CO_2, and left qualitatively intact the responses to injections of histamine and phenyl diguanide (which stimulate lung irritant and type-J receptors. Pneumothorax caused a greater prolongation of inspiratory time during pulmonary stretch receptor block than in controls. It is concluded that lung irritant and type-J receptors change the durations of inspiration and expiration but not the rate of build-up of phrenic discharge during inspiration.

RESUME

Nous avons étudié les actions des réflexes d'origine pulmonaire chez le lapin dont l'activité des récepteurs d'étirement du poumon est déprimée par l'inhalation de 200 p.p.m. de SO_2. Les réponses obtenues ont été comparées avec celles des animaux témoins et celles d'animaux vagotomisés. Le blocage des récepteurs d'étirement du poumon a augmenté la réponse ventilatoire des lapins au CO_2. Ce blocage ne modifie pas qualitativement les réponses aux injections d'histamine et de phényl diguanide (qui stimulent les récepteurs d'irritation et les récepteurs de type J). Le pneumothorax a entraîné une prolongation du temps inspiratoire plus marquée chez les animaux dont les récepteurs d'irritation sont bloqués que chez les animaux témoins.

On peut ainsi déduire que les récepteurs d'irritation et de type J changent les durées de l'inspiration et d'expiration mais ne modifient pas la pente de l'activité électrique intégrée de la décharge phrénique pendant l'inspiration.

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Publication 10.

Wise.J.C.M. & Davies,A.(1976)

A simple sulphur dioxide meter.

Biological and Medical Engineering, 14(5).545-547.

A simple sulphur-dioxide meter*

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Abstract—*A simple sulphur-dioxide meter is described which has been used in a study of experimental respiratory pathology. The method is based on the changes in electrical conductivity produced by the reaction of sulphur dioxide with a solution of hydrogen peroxide.*

Keywords—*Experimental pathology, Sulphur-dioxide meter*

Introduction

FOR MANY years, sulphur dioxide has been recognised as an important constituent of polluted air. Apart from its importance to public health, it has been used as an experimental tool in the production of lung disease and in the investigation of reflexes from the lungs (DALHAMN and STRANDBERG, 1961; GOLDRING *et al.*, 1967; ALARIE *et al.*, 1972; DIXON, 1975).

Many methods of measuring the concentration of sulphur dioxide exist, most of them based on colour changes in solutions or pastes. The most widely used method is that of WEST and GAEKE (1956), which involves detecting a colour change with formaldehyde and acid-bleached pararosanaline. The British Standard method for determination of sulphur dioxide (BRITISH STANDARDS INSTITUTION, 1969) depends on the measurement of the net acidity resulting from its absorption in hydrogen-peroxide solution and by titration to pH 4·5 with standard alkali.

This method has been used in modified forms to measure sulphur-dioxide concentration by conductimetric methods (NASH, 1961; KILLICK, 1969) and provides the basis of the present method.

The piezoelectric detector of KING (1965) is useful for measuring the concentration of a number of gases and has been developed by FRECHETTE and FASCHING (1973) as a method of measuring sulphur dioxide.

Most of these methods are useful in specific circumstances but are time consuming, lack ruggedness or are troubled by chemical interference. The device we describe uses the well known effect of a change in conductivity of hydrogen-peroxide solution produced by reaction with sulphur dioxide, and utilises the high-gain properties of modern solid-state amplifiers. The mode of operation of the instrument will be easily understood by reference to the block and circuit diagrams (Figs. 1 and 2). 15 ml of hydrogen-peroxide solution are drawn into a 100 ml glass syringe. In the syringe the solution makes contact with a pair of electrodes. The conductivity cell so formed is excited by a 3·3 kHz sine wave through a constant-current amplifier. Changes in conductivity therefore result in proportional changes in the potential between the electrodes.

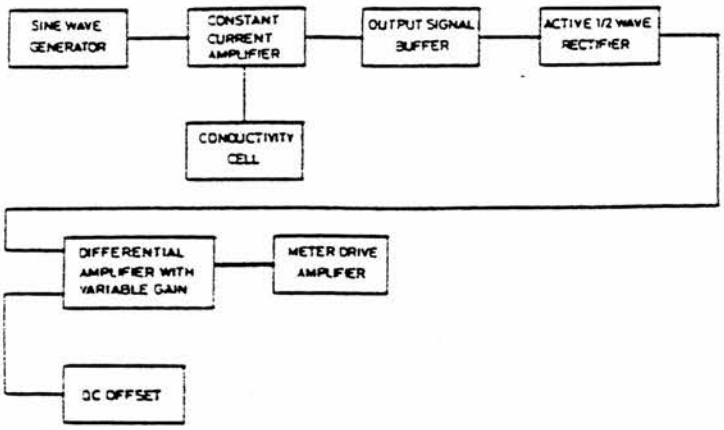

Fig. 1 SO_2 meter block diagram

*First received 15th September and in final form 8th December 1975

This is the principle on which the instrument works. A meter zero is first obtained, with sulphur-dioxide-free solution in the cell, by adjusting a d.c. offset acting on one input of an operational amplifier. To prevent current drain, the cell is isolated by an operational amplifier in a buffer mode. The other input of the amplifier in differential mode is provided by the halfwave rectified signal from the cell. A known volume of known concentration sulphur dioxide* is drawn into the cell through the hydrogen peroxide by a narrow needle. This calibrating gas is of the same order of concentration as the unknown gas and the meter reading is adjusted to the calibrating value by altering the differential amplifier gain. The meter is now calibrated, and if the same volume of unknown concentration sulphur dioxide is

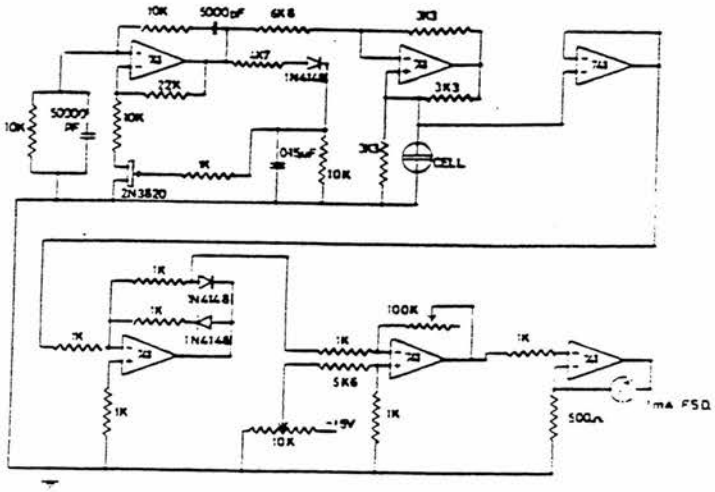

Fig. 2 SO_2 meter circuit diagram

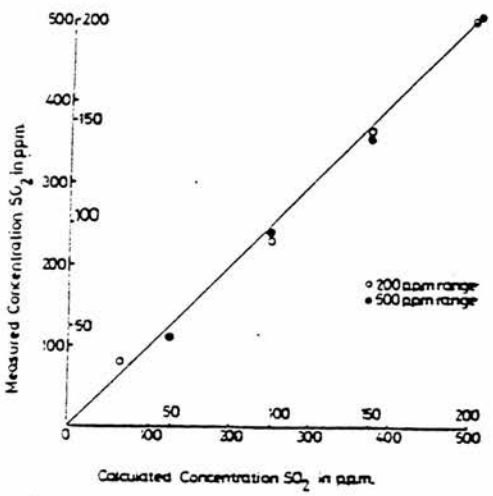

Fig. 3 SO_2 meter calibration
The ordinate represents the reading obtained from the meter when sulphur-dioxide mixtures, whose calculated concentration is shown on the abscissa, were sampled. The concentration of the mixtures was altered by dilution with air

drawn through a fresh 15 ml aliquot of hydrogen peroxide it will produce a signal, proportional to sulphur-dioxide concentration, which in turn produces a deflection on the meter giving a direct measure of sulphur-dioxide concentration. A driver amplifier in association with the meter ensures that no current is drawn from the active part of the circuit.

While designed to operate at up to 500 parts in 10^6 we have been most interested in concentrations below 200 parts in 10^6. Over both ranges the relationship between concentration and meter deflection is rectilinear.

Fig. 3 shows the results of measurements of 500, 375, 250 and 125 parts in 10^6 sulphur dioxide prepared by diluting 500 parts in 10^6 sulphur dioxide with air in a glass syringe; 200, 150, 100 and 25 parts in 10^6 were prepared in a similar manner.

Titrametric measurements of the sulphur-dioxide content of these mixtures were not made, and the limits of accuracy of dilution probably contributed to the small departures from the line of identity. The individual results were highly repeatable, the standard error of the mean in ten consecutive samples of 28·3 parts in 10^6 sulphur dioxide being $\pm 1 \cdot 1$.

*From British Oxygen Company Special Gases

It can be seen from the description of the instrument that by altering the gain and the volume of the gas sampled the range can be adjusted. We have been interested in concentrations used in experimental pathology (usually 100–200 parts in 10^6). At low levels of sulphur dioxide it may be necessary to acidify the hydrogen peroxide to minimise possible interference from carbon dioxide (NASH, 1961).

Acknowledgments—The authors thank D. Richie for his invaluable advice on the design of the circuit. One of us (A.D.) was supported by the Medical Research Council during the work.

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Un simple appareil de mesure à l'anhydride sulfureux

Sommaire—On décrit un simple appareil de mesure à l'anhydride sulfureux qui a été utilisé pour une étude de la pathologie respiratoire experimentale. Cette méthode est basée sur les modifications de la conductivité électrique provoquées par la réaction de l'anhydride sulfureux avec une solution d'eau oxygenée.

Ein einfaches Meßgerät für Schwefeldioxid

Zusammenfassung—Es wird ein einfaches Gerät zum Messen von Schwefeldioxid beschrieben, das zu einer Untersuchung der experimenteilen Atmungspathologie eingesetzt wurde. Das Verfahren fußt auf Änderungen in der elektrischen Leitfähigkeit, die durch die Reaktion des Schwefeldioxids mit einer Lösung des Wasserstoffsuperoxids entsteht.

Publication 11.

Davies,A. Dixon,M. & Widdicombe,J.G.(1976)

Lung reflexes in rabbits with inhibition of pulmonary stretch receptor activity.

J. Physiol. 260, 36-7p, 1976.

Lung reflexes in rabbits with inhibition of pulmonary stretch receptor activity

By A. Davies, M. Dixon and J. G. Widdicombe. *Department of Physiology, St George's Hospital Medical School, Tooting, London SW17 0QT*

The pattern of breathing and the relationship between tidal volume, inspiratory duration and expiratory duration have recently been investigated in cats (Euler, Herrero & Wexler, 1970; Clark & Euler, 1972; Grunstein, Younes & Milic-Emili, 1973; Bradley, Euler, Marttila & Roos, 1974; Widdicombe & Winning, 1974). Hyperthermia and hypercapnia were used to change the pattern of breathing, and the changes were explained in terms of the inflation reflex generated by the activity of pulmonary stretch receptors.

We have used anaesthetized rabbits, before and after inhibiting stretch receptor activity, and stimulated lung irritant and J-receptors in order to determine the role of the groups of receptors in modifying the pattern of breathing. Breathing and lung mechanics were recorded. Integrated phrenic multifibre discharge was taken as an index of the inspiratory motor output of the brain-stem respiratory complex. Lung reflexes were produced by injections of histamine and phenyl diguanide, and by pneumothorax. The response to inhaled carbon dioxide was also studied. Pulmonary stretch receptor activity was inhibited peripherally by causing the animals to breath 200 p.p.m. SO_2 for 10 min (Callanan, Dixon & Widdicombe, 1975). The lung reflexes were elicited again and compared with the responses when the stretch receptors had been intact.

Most reflexes persisted in a modified form after the inhibition of stretch receptor activity. The ventilatory response to CO_2 was enhanced. For the reflexes generated from the lungs which affected the pattern of breathing, the rate of increase of phrenic activity was influenced neither by the presence or absence of activity in pulmonary stretch receptors nor by the reflexes from other lung receptors; only the duration of the phrenic bursts of activity was changed. Increased carbon dioxide concentration in the inspired air on the other hand increased the rate of increase of phrenic activity.

Our results can be interpreted in terms of two systems controlling the nature of individual breaths. One, central, governs the rate of increase of inspiratory activity. The other, from the lung reflexes studied, modifies only the duration of this activity.

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Publication 12.

Davies,A. Roumy.,M.(1977)

Changes in the pattern of breathing provoked by irritant receptors.

J. Physiol. 272 (30), 78-79p.

Changes in the pattern of breathing provoked by irritant receptors

BY A. DAVIES and M. ROUMY. *St George's Hospital Medical School, Tooting, London SW17 0RE.*

The exact role of lung irritant receptors in the control of breathing is still far from clear. However, they probably exert strong effects on the pattern of breathing and may play an important part in its control in normal and certain disease states (Widdicombe, 1971). Investigators have been able to produce diametrically opposite effects on breathing by stimulating irritant receptors in different ways; thus it is claimed that they can cause deep augmented breaths (Sellick & Widdicombe, 1970) and also that they can cause rapid shallow breathing (Widdicombe & Winning, 1976). In addition, after pulmonary stretch receptor paralysis by SO_2 the pattern of breathing produced by the introduction of a pneumothorax is quite different from that produced by its withdrawal (Davies, 1976), despite the fact that both procedures stimulate irritant receptors to about the same degree. Reynolds (1962) observed a refractory period after the inspiratory-augmenting reflex which he provoked in cats by the sinusoidal ventilation of their lungs at large tidal volumes, a procedure which would repeatedly stimulate irritant receptors. Such refractoriness might explain some of the apparent discrepancies.

To investigate the reflex effects of irritant receptors further we provoked intense irritant receptor activity in anaesthetized rabbits by briefly inflating or deflating their lungs with short (100 ms) pulses of positive or negative pressure. Irrespective of the sign of the pressure, the effect of a single pulse was invariably a lengthening of inspiration and a shortening of expiration in the breath containing the pulse. The sensitivity of the animal to the pulse varied, being greatest when the pulse was at the beginning of inspiration and falling to zero at the end of inspiration.

For about 30 breaths after the response to a single pulse, subsequent pulses did not lengthen inspiration, but caused only decreases in the length of expiration. A similar refractory period was seen to follow spontaneous augmented breaths.

These findings explain our previous observations on the pattern of breathing in pneumothorax; induction of pneumothorax causes a prolonged inspiration but removal of the pneumothorax does not, presumably because the inspiratory-augmenting reflex is refractory in the latter instance. The results support an inspiratory-augmenting role for irritant receptors and show the existence of independent systems controlling the duration of inspiration and expiration.

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Publication 13.

Davies,A. Roumy, M., Widdicombe,J.G. &
Wise,J.C.M.(1977)

The effect of brief changes in lung volume on pattern of breathing in rabbits.

In: Proc. International Union of Physiological Sciences (Paris).Vol. 13, 163p.

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EFFECT OF BRIEF CHANGES IN LUNG VOLUME ON PATTERN OF BREATHING IN RABBITS.

by: A. Davies, M. Roumy, J.G. Widdicombe and J.C.M. Wise.
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Pulmonary stretch receptors have been strongly implicated in the control of the pattern of breathing. Their activity inhibits inspiratory efforts, and prolongs expiration. The influence of rapidly adapting irritant receptors is not so clear and we have investigated this using a method of paralysing stretch receptors while leaving irritants intact. Pentobarbitone anaesthetised rabbits had their lungs subjected to brief (100ms.) pulses of inflation and deflation at various times in the respiratory cycle, both before and after paralysing their pulmonary stretch receptors with sulphur dioxide. The pulses stimulated irritant receptors as shown by recording from the vagus. The inflation or deflation pulses usually changed the pattern of breathing, the sensitivity depending on the phase of the respira tory cycle containing the pulse. Expiration was consistently shortened. The inspiratory effort usually increased, irrespective of whether the pulse was of inflation or deflation, which could not be repeated for several breaths after the original response. This refractoriness did not apply to the shortening of expiration. The results illustrate the effect of irritant receptors on the pattern of breathing.

Publication 14.

Davies,A & Roumy,M.(1978)

The inspiratory augmenting effects of lung irritant receptor activity.

J. Physiol. 275, 14p.

The inspiratory augmenting effect of lung irritant receptor activity

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Various experimental methods have been used to separate and stimulate the different receptor systems of the lungs. Pulses and steps of inflation and deflation have been used to modify the pattern of breathing in man and experimental animals and the results have been explained in terms of modified lung stretch receptor activity (Euler, Herrero & Wexler, 1970; Clark & Euler, 1972; Bradley, Euler, Martila & Roos, 1974). We have observed that 200 p.p.m. gaseous sulphur dioxide paralyses lung stretch receptors in anaesthetized rabbits (Davies, Dixon & Widdicombe, 1976). This results in changes in the pattern of breathing, inspiratory time being lengthened, tidal volume increased and expiratory time shortened. We attribute this pattern partly to the remaining action of lung irritant receptors, which are not paralysed by sulphur dioxide. In animals with stretch receptors paralysed, lung reflexes are modified due to the dominant activity of irritant receptors which also probably play an important role in normal breathing and in the modified patterns seen in lung disease. However, the precise action of the lung irritant receptors on breathing is controversial. Different methods of stimulating irritant receptors have produced widely differing changes in the pattern of breathing (Sellick & Widdicombe, 1970; Widdicombe & Winning, 1976; Dain, Boushey & Gold, 1975) and until now it has been difficult to assign a consistent role to these receptors.

We have taken rabbits anaesthetized with sodium pentobarbitone (40 mg kg^{-1}), before and after paralysing their lung stretch receptors with sulphur dioxide, and applied brief pulses of inflation and deflation to their lungs. This procedure has been shown to stimulate irritant receptors, and it produced increases in inspiratory time and decreases in expiratory time, irrespective of whether the pulses were of inflation or deflation or whether stretch receptors were intact or paralysed. The changes in expiratory time could be produced repeatedly, but the changes in inspiratory time were refractory for about 2 min. This refractory period explains many of the contradictory results obtained in previous experiments involving irritant receptor stimulation. The inspiratory augmenting effect and its refractory period was demonstrated.

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Publication 15.

Davies,A. (1977)

The effect of lung irritant receptor activity on expiratory time.

J. Physiol. 275, 39-40p.

The effect of irritant receptor activity on expiratory time

BY A. DAVIES. *Department of Physiology, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE*

In previous communications we have discussed the role played by stretch receptors in the control of inspiratory time (t_I) in anaesthetized rabbits (Davies, Dixon & Widdicombe, 1976; Davies, 1976). We have also demonstrated the ability of intense irritant receptor stimulation to provoke an augmented breath containing an extended t_I (Davies, Roumy, Widdicombe & Wise, 1977). The effect of such stimulation during the inspiratory phase was to reduce the following expiratory time (t_E).

To investigate further the effect of irritant receptor activity on t_E we have recorded the pattern of resting and CO_2-stimulated breathing in rabbits (anaesthetized with sodium pentobarbitone, 40 mg/kg) before and after lung stretch receptor paralysis by SO_2 and before and after vagotomy. We also provoked lung reflexes by deflating the lungs of anaesthetized rabbits with negative pressures of -5 cm H_2O for about 10 breaths both before and after stretch receptor paralysis, and recorded the patterns of breathing.

The role of stretch receptors in the control of t_I was clearly seen in the shift of the t_I/V_T plot produced by their paralysis. On the other hand, the plot of t_E/V_T lay on the same line before and after stretch receptor paralysis, provided the vagi were intact, indicating that control of t_E was not dominated by stretch receptor activity. When changes in irritant receptor activity were produced by negative pressure deflations, t_E was shortened whether stretch receptors were intact or paralysed.

We therefore suggest that in quiet breathing in these experimental conditions the control of inspiration is dominated by lung stretch receptor activity and the control of expiration by lung irritant receptor activity.

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Davies,A.,Dixon,M.,Widdicombe,J.G., &
Wise,J.C.M.(1978)

Lung stretch receptor paralysis by sulphur dioxide.

J.Physiol. 275, 13P.

Lung stretch receptor paralysis by sulphur dioxide

By A. Davies, M. Dixon, J. G. Widdicombe and J. C. M. Wise. *Department of Physiology, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE*

It has long been accepted that the pattern of breathing of an animal is modified by vagal afferent activity from receptors in the lungs. However, there are three major groups of lung receptor and the relative roles of the three groups are controversial. The first group consists of stretch receptors, whose existence was postulated by Hering & Breuer (1868) and whose activity was first recorded by Adrian (1933). The second group is of rapidly adapting 'irritant' receptors which have been extensively investigated by several groups of workers (Widdicombe, 1974; Paintal, 1973). The J-receptors discovered by Paintal (1973) form the third group of lung receptors but these are thought not to be active in normal quiet breathing. We have observed that pulmonary stretch receptor activity and the Hering–Breuer inflation reflex can be abolished in anaesthetized (sodium pentobarbitone, 40 mg/kg) rabbits by causing them to breath 200 p.p.m. sulphur dioxide for 10 min, while the activity of irritant receptors is retained. We have used this method to separate the contribution made by irritant receptors from that of stretch receptors to the pattern of breathing (Davies & Roumy, 1978) and to lung reflexes (Davies, Dixon & Widdicombe, 1976), many of which persist in modified form after the inhibition of stretch receptor activity.

In this demonstration the activity of single fibres from individual stretch and irritant receptors in anaesthetized rabbits during and after exposure to sulphur dioxide was displayed, together with concomitant changes in the animals' pattern of breathing.

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Publication 17.

Davies,A.& Wise,J.C.M.(1978)

A true integrator with automatic reset.

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True integrator with automatic reset

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Abstract—*A simple electronic integrator is described which has been used to study respiratory activity in experimental animals. It provides a useful advance on previous 'leaky' integrators without resort to the expense and complexity of computing techniques.*

Keywords—*Integrator, Nerve activity*

1 Introduction

TENSION in mammalian skeletal muscles is usually provoked by bursts of activity in the motor nerves running to them. This activity consists of a rapid series of impulses, action potentials. The nerve activity may be continuous, regular or irregular, as in the case of most skeletal muscle, or cyclical, as in the case of respiratory muscles. The high frequency of discharge and the fact that frequency is continually changing makes this activity difficult to analyse.

We have been particularly interested in analysing the cyclic activity in the phrenic nerve which causes contraction of the diaphragm (and thus inspiration). A convenient way of displaying this activity is in the form of its integral.

Until fairly recently the state of electronic components was such that it was difficult to construct an integrator whose output did not drift erratically. To overcome this problem workers have resorted to 'leaky' integrators in which the activity being recorded counteracted the tendency of the integrator to return to zero output. The disadvantage of this system is that the shape of the output signal depends both on the electronic characteristics of the instrument and the pattern of the input discharge and this results in an output only amenable to qualitative analysis. An advantage of our integrator system is that the output returns to zero at the end of each period of activity. An ideal system for recording the type of biological activity we have described would provide a true integral and automatically reset at the end of each burst of activity (BARTOLI *et al.*, 1972). We have built such an integrator and used it to investigate activity in the phrenic nerve of rabbits under various experimental conditions (DAVIES, 1976).

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2 Mode of operation

The mode of operation of the instrument may be considered in four main parts. The raw signal is pretreated by the first part comprising buffer amplifier A, the gain control circuit and the fullwave rectifying amplifier. This part of the circuit converts the signal into a form suitable for the true integrator, the second part of the circuit, which provides the signal for the output amplifier of the instrument. To prevent the true integrator saturating, a voltage comparator C triggers the last part of the circuit, the monostable and f.e.t. switch which resets the integrator. The periodic reset facility stems from the third part of the circuit, the leaky integrator and comparator E.

3 Circuit description

The activity to be measured consisted of a series of short pulses of various amplitudes (Fig. 1*a*) and the way in which the instrument monitored this can be understood by reference to the block and circuit

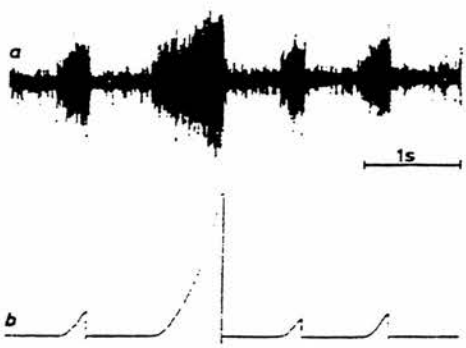

Fig. 1 Activity of phrenic nerve
(a) *Activity of phrenic nerve of rabbit*
(b) *Integral of this activity*

diagrams (Figs. 2 and 3). The signal to be integrated is applied to the input which is a.c. coupled to a buffer amplifier A. The output from the buffer is fed to a switchable resistor network that provides variable attentuation of the signal amplified by a second noninverting amplifier B with tenfold gain. This amplifier has a d.c. shift control on the inverting input to enable the output to be trimmed to allow the full wave rectifying amplifier to output equal absolute values for signals of equal magnitude but opposite sign. The rectifying amplifier also has a shift control to enable the output signal to be trimmed to sit on zero volts because not only is the signal amplified by the zero reset amplifier, but also it is summed by the integrator and both these operations require the least possible interference from standing voltages. The voltage shift control of the zero reset amplifier enables it to be set on a small negative d.c. level. This level forces the output from the 'leaky' integrator to be negative with the signal present and positive when it is absent. In choosing the time constant for the leaky integrator (150 μs) we have taken into account the magnitude of its output and the need for a rate of leak which will reset the true integrator before the next burst of input activity. This small voltage swing is fed into a high gain amplifier E, the output of which makes a large negative-to-positive swing on cessation of the signal. This is fed into the inverting input of an operational amplifier in differential mode D.

The other input to this amplifier is obtained by feeding the output of the true integrator into the noninverting input of differential amplifier C. The inverting input is biased at -6 V. This amplifier has high gain and if the level of the integral is less than six volts its output is positive swinging rapidly to negative when the integral exceeds that voltage. This signal is then fed through a diode to differential amplifier D so that the noninverting input senses a zero-to-negative swing as the integral exceeds the preset value. Consequently, if the signal is less than 6 V and still firing, then the inverting input is negative and the noninverting input is zero, forcing the output to be positive. If the signal stops the inverting input experiences a negative-to-positive transient, which, with the noninverting input still at zero volts, causes the output to swing positive-to-negative. If the signal is firing and the integral exceeds the 6 V level, then the inverting input is negative while the noninverting input experiences a zero-to-negative transient. However, the resistor between the inverting input of the amplifier and ground halves the voltage swing, and although the inverting input is negative, the zero-to-negative swing at the noninverting input causes it to become more negative than the inverting input and forces a positive-to-negative swing at the output. If the signal stopped firing at exactly the same time as the integral reached the preset voltage then the inverting input would swing positively but the noninverting input would swing negatively. This would force the output from positive to negative. This trigger differential amplifier signal is differentiated and the positive pulse from the differential is shunted to

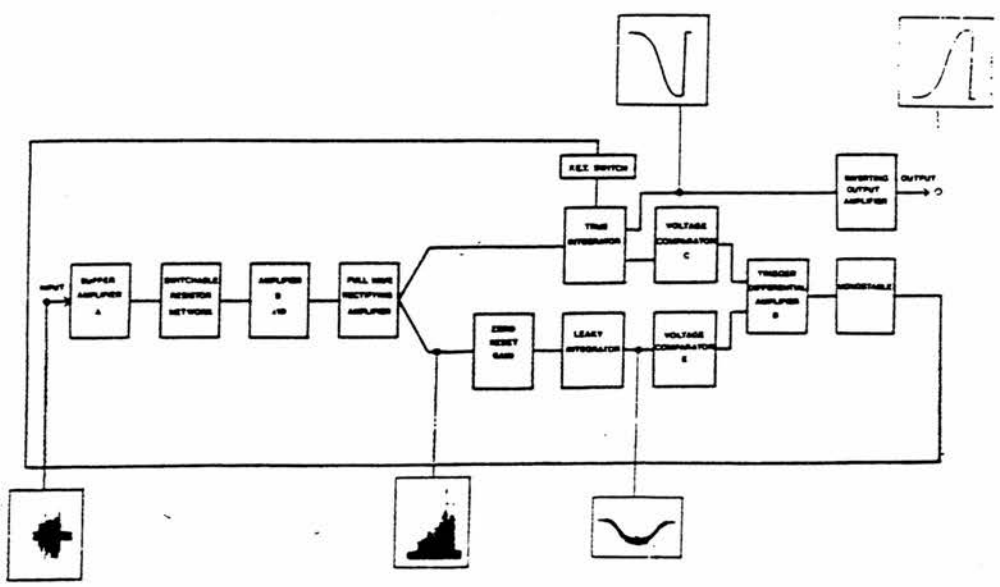

Fig. 2 Integrator

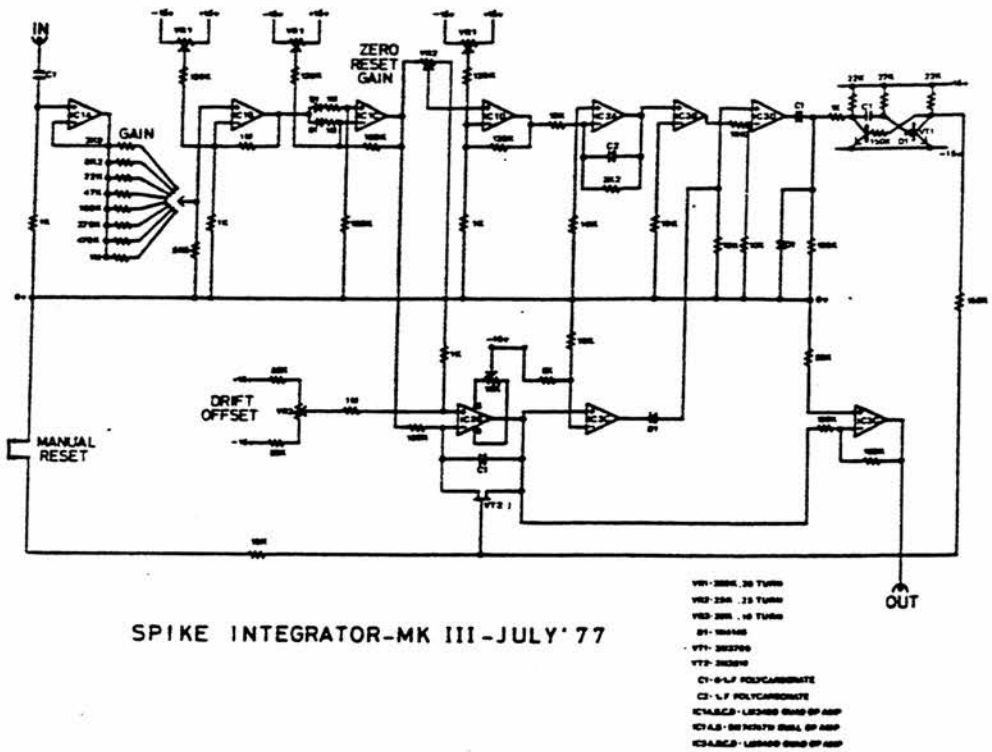

Fig. 3 Integrator circuit diagram (output of each main section is shown in the rectangles)

ground through a diode. This produces a pulse only when the amplifier swings from positive to negative and not when it returns to the resting state. This pulse is applied to a discrete component monostable producing a negative-to-positive pulse of 1 ms duration which is fed to the f.e.t. switch across the integrating capacitor. This allows the capacitor to discharge and the integral returns to zero causing the trigger differential amplifier D to return to its resting state. The output of the instrument is a rising signal which continues until the input activity ceases. The output then falls to zero and the instrument is ready to integrate the next burst of activity (Fig. 1*b*).

Fig. 4 shows a plot of the slope of the integrator output against the input frequency of a series of 3 V, 0·2 ms spikes of various frequencies produced by a Devices stimulator, demonstrating the linearity of the instrument.

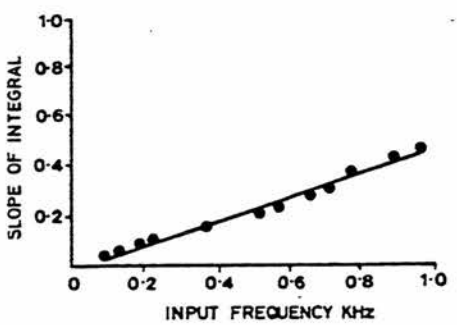

Fig. 4 Slope of the integrator (ordinate) plotted against the frequency of a 2V input signal (abscisa)

3 Summary

Other workers (WIEMER *et al.*, 1975) have reviewed methods of evaluating neural activity. Most of these involve the use of sophisticated electronic computer techniques (WIEMER *et al.*, 1975). The instrument we describe offers advantages of simplicity and economy in the measurement of activity of biological or mechanical systems whose output consists of cyclical bursts of discrete pulses.

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Un intégrateur vrai avec réenclenchement automatique

Sommaire—Un intégrateur électronique simple utilisé pour étudier l'activité respiratoire chez les animaux expérimentaux est décrit. Il constitue une amélioration utile par rapport aux intégrateurs 'à fuites' sans avoir à utiliser de techniques informatiques coûteuses et complexes.

Ein echter Integrator mit automatischer Rückstellung

Zusammenfassung—Es wird ein einfacher elektronischer Integrator beschrieben, der zur Untersuchung der Atemtätigkeit bei Versuchstieren verwendet wurde. Er stellt einen nützlichen Fortschritt gegenüber den bisherigen 'undichten' Integratoren dar, ohne kostspielige und komplizierte Rechenverfahren zu erfordern.

Publication 18.

Davies,A.,Dixon,M.,Callanan,D.,Huszczuk,A.,
Widdicombe ,J.G.& Wise,J.C.M.(1978).

Lung reflexes in rabbits during pulmonary stretch receptor block by sulphur dioxide.

Respir. Physiol. 34, 83-101.

Respiration Physiology (1978) 34, 83–101

LUNG REFLEXES IN RABBITS DURING PULMONARY STRETCH RECEPTOR BLOCK BY SULPHUR DIOXIDE*

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Abstract. Anaesthetized rabbits were given 200 ppm sulphur dioxide to breathe for 10 min. This abolished activity in 23 of 26 pulmonary stretch receptors, while leaving that of lung irritant receptors unimpaired. The Breuer-Hering reflex was abolished and breathing became deeper and slower. Inspiratory time (T_I) was increased and expiratory time (T_E) decreased. Subsequent vagotomy increased tidal volume (V_T), T_I and T_E. In animals with stretch receptors blocked, injections of phenyl diguanide and histamine still increased breathing frequency and decreased V_T, indicating that reflexes from lung irritant and J-receptors were intact. Inhalation of 8% CO_2 caused a bigger increase in frequency and tidal volume in rabbits with stretch receptor block compared with controls or those after vagotomy. Induction of pneumothorax with stretch receptor block transiently prolonged T_I and shortened T_E; removal of the pneumothorax also transiently shortened T_E and usually also decreased T_I. The results suggest that lung irritant receptors reflexly shorten T_E in all our experimental conditions, but have various effects on T_I which may depend on the timing of the irritant receptor discharge and refractoriness of the inspiratory response.

Irritant receptor — Sulphur dioxide
Lung reflexes — Vagal reflexes
Pattern of breathing

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The effect of the Breuer-Hering reflex on the pattern of breathing has been studied quantitatively and extensively in recent years (reviewed by Bradley, 1977). Other lung reflexes, in particular from lung irritant and alveolar J-receptors, have been far less studied, and very few papers analyse the changes in pattern of breathing quantitatively (Karczewski, 1975; Winning and Widdicombe, 1976). These investigations of lung irritant and J-reflexes suffer from two main defects: most of the stimuli used affect more than one group of lung receptors; and as soon as a respiratory response starts it is secondarily modified by activity from pulmonary stretch receptors. Therefore it is difficult to be sure of the primary reflex action on breathing of lung irritant and J-receptors.

We have recently developed a method whereby the natural activity of pulmonary stretch receptors in rabbits is blocked, and with this preparation it is possible to stimulate lung irritant and J-receptors without appreciable modification of the response by pulmonary stretch receptors. The specificity of the differential vagal block is probably greater than that of other methods, such as cold or DC current applied to the vagus nerves (see Discussion). Brief descriptions of the method and some results have been published (Callanan *et al.*, 1975; Davies, 1976; Davies *et al.*, 1976).

Methods

New Zealand white rabbits weighing from 2.5 to 3.5 kg were anaesthetized with an intravenous injection of 30 mg/kg sodium pentobarbitone and prepared for recording of lung mechanics and patterns of breathing. Catheters were tied into the right femoral artery and vein. Supplementary doses of pentobarbitone were given to maintain surgical anaesthesia. Airflow was measured by a Fleisch pneumotachograph head connected to a tracheal cannula. Tidal volume was obtained by integrating flow electronically. End-expiratory volume was assessed from the change in level of the tidal volume record (see Discussion). Transpulmonary pressure was measured by a differential capacitance manometer connected between a lower right intercostal space and the trachea. The airflow, tidal volume and transpulmonary pressure signals were used to determine total lung resistance and compliance by the subtractor method (Mead and Whittenberger, 1953) as modified by Nadel and Widdicombe (1962). Carbon dioxide was monitored by an infra-red gas analyser (Beckman L.B.1), sampling at 500 ml/min from the rostral side of the pneumotachograph head, so that signals from the latter were not distorted.

Both vagus nerves were exposed in the mid-cervical region and loops of silk thread were placed round them. In those experiments where action potentials were recorded from the vagus the right vagus nerve was cut high in the neck and the cut end was placed in a copper tray filled with liquid paraffin. 'Single fibre preparations' were made and placed on a pair of platinum electrodes. The electrical activity of the nerve was amplified by a high-gain RC amplifier (Tektronix 122), displayed on an

oscilloscope and recorded on magnetic tape (Ampex SP300). Phrenic nerve activity was recorded from multifibre strands dissected out under liquid paraffin from the cut uppermost root of the right phrenic nerve. The multi-fibre signal was rectified and then integrated with respect to time. The integrator was not of the conventional 'leaky' RC type, but instead provided a true integral of phrenic activity, and so did not have a time constant in the conventional sense. The trace was zeroed automatically at the end of each phrenic discharge. The integrated phrenic record was analysed by measuring its maximum height (H) and its average slope (S). The relationship between phrenic height and tidal volume is complex, since it depends on changes in lung compliance, the linearity of the lung compliance curve and changes in end-expiratory volume. This complexity may explain the lack of correlation between phrenic height and tidal volume in some of our results (*e.g.* tables 2, 3, and 5).

Sulphur dioxide mixtures were made up in a polyethylene Douglas bag by mixing air and pure SO_2 (BOC Special Gases) from two rotameters. The concentration of SO_2 was measured using a Dragor Normalar gas sampling system. For administration of SO_2, a stream of the gas mixture was drawn across the rabbit's trachea by a suction pump, adjusted so that the pressure at the tracheal cannula was atmospheric, the pressure in the Douglas bag being slightly positive. Carbon dioxide was administered by passing calibrated mixtures of the gas in air across the tracheal cannula until a steady state of breathing was achieved, judged by a near-constant value of minute volume. Pneumothorax was induced by the rapid injection of 40 ml of air into the pleural cannula. The air was gently withdrawn to terminate pneumothorax. Histamine acid phosphate (Evans Medical Ltd.) and phenyl diguanide hydrochloride (H and K Laboratories Inc.) were made up in 0.9% saline and administered via the femoral venous catheter in doses of 100 μg of the salts.

In animals paralysed while single-fibre recordings were being made, 60 mg of gallamine triethiodide (Flaxedil: May and Baker) were given intravenously. The animal was then artificially ventilated with a pattern which kept end-tidal CO_2% close to the spontaneous breathing level. In these animals supplementary doses of anaesthetic were administered at the same rate as before paralysis.

Results

Pattern of breathing

Sulphur dioxide was given in a concentration of 200 ppm in air for 10 min. On first introduction to the gas the animals usually coughed. Within the first minute of exposure there was a vagally-mediated bronchoconstriction in which total lung resistance approximately doubled. By the end of the 10-min exposure, total lung resistance was only slightly higher than the control value (control 27.5 $\pm$ 1.4, end of exposure 31.4 $\pm$ 1.7 cm $H_2O \cdot l^{-1} \cdot s$; means and standard errors, n = 46 tests in 46 rabbits. NS); compliance was not significantly changed by exposure to SO_2 (control

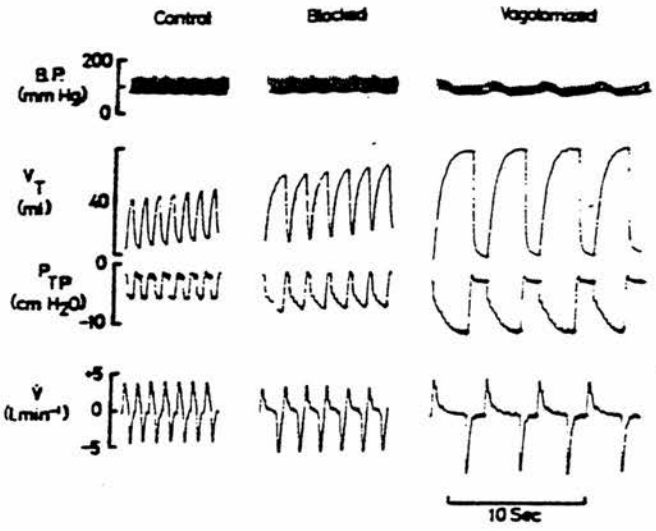

Fig. 1. Effect of stretch receptor block by SO_2 and vagotomy on, from above downwards: blood pressure, tidal volume (with some integrator drift), transpulmonary pressure and airflow. Records fr. spontaneously breathing rabbit.

TABLE I
Changes in pattern of breathing due to stretch receptor block and vagotomy

	Control	Receptor block	Vagotomized
Inspiratory time t_I (s)	0.44 ± 0.05	0.80 ± 0.15**	1.2 ± 0.42**
Expiratory time t_E (s)	0.79 ± 0.13	0.60 ± 0.11**	1.05 ± 0.20**
Tidal volume V_T (ml)	18.8 ± 3.5	23.0 ± 4.0**	31.5 ± 5.3**
Phrenic height H (mm)	23.0 ± 12.0	47.0 ± 4.0	57.0 ± 4.9*
Phrenic slope S (°)	27.6 ± 8.0	30.5 ± 14.0	35.3 ± 11.6

Significance of differences from controls: * $P < 0.05$; ** $P < 0.01$, by sign test; n = 25 for t_I, t_E and V_T. n = 7 for H and S.

7.13 ± 0.5, end of exposure 7.0 ± 0.5 ml · cm H_2O^{-1}; n = 46, NS). There was always a slowing of breathing (fig. 1). This slowing was due to an increase in inspiratory time (t_I) which was greater than the slight reduction in expiratory time (t_E) (table I). Tidal volume (V_T) was increased. Exposure to SO_2 increased the duration and maximum height of phrenic discharge but did not significantly affect the slope of the phrenic integral. After removal of SO_2, the pattern of breathing returned to control values in about 20–60 min.

Bilateral cervical vagotomy, like the administration of SO_2, increased t_I and V_T; t_E on the other hand increased to a value significantly greater than in the control. In 5 out of 5 rabbits after vagotomy, administration of SO_2 had no significant effects on pattern of breathing or on phrenic discharge.

Vagotomy, like exposure to SO_2, significantly increased the duration and maximum height of the phrenic integral, and increased the slope of the phrenic integral but not statistically significantly.

Receptor activity

To analyse the cause of the change in pattern of breathing induced by SO_2, we recorded single-fibre activity from pulmonary stretch and lung irritant receptors in bilaterally vagotomized rabbits.

Receptor activity was studied both in paralysed artificially-ventilated rabbits, in which tidal volume would be approximately constant, and in spontaneously breathing rabbits in which tidal volume and breathing frequency might change. Twenty-three of the twenty-six slowly adapting pulmonary stretch receptors studied in paralysed ventilated rabbits were completely bocked by 1–8-min exposure to 200 ppm SO_2 (fig. 2). The activity of the three fibres not completely blocked was clearly depressed (by about half). For ten of the fibres completely blocked by SO_2, activity gradually decreased until the fibres became silent; five fibres were abruptly silenced while eight showed an increase in activity before silencing. Changes in the pattern of firing were often observed before complete inhibition of activity. Fibres firing only in inspiration usually gained expiratory activity and others lost their phasic activity and became tonically active. When the fibres had been silenced by SO_2 during spontaneous breathing, large positive pressure inflations of the lungs always failed to elicit any activity.

All the pulmonary stretch receptors recovered their spontaneous activity, quantitatively to control values, within 20–60 min of removal of the SO_2.

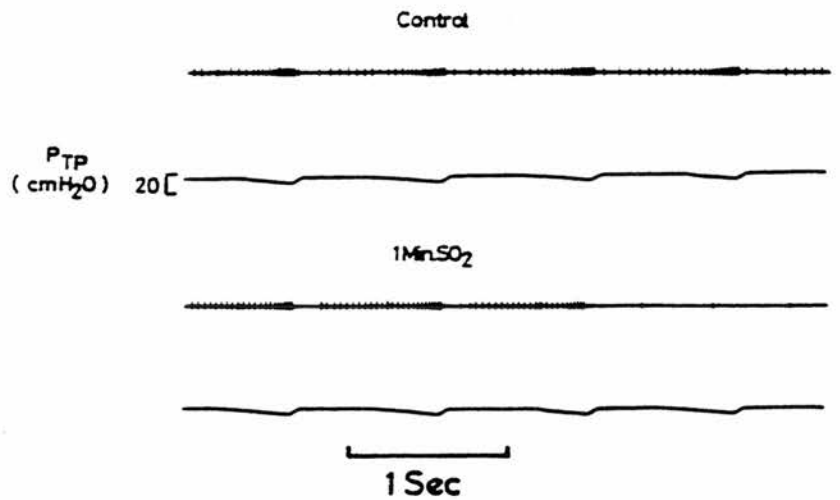

Fig. 2. Effect of 200 ppm SO_2 on the activity of a pulmonary stretch receptor in a bilaterally vagotomized, paralysed rabbit. The traces show a single-fibre discharge recorded from the vagus nerve and transpulmonary pressure. Note the complete cessation of activity in the lowest record.

We tested whether inhalation of an alkaline gas, ammonia, might also inhibit pulmonary stretch receptors. When 2,200 ppm ammonia were added to the inspired air, six out of six receptors in three paralysed (constant pump volume) bilaterally vagotomized animals clearly increased their activity. Two out of two stretch receptors in a nonparalysed unilaterally vagotomized animal increased activity, and the rabbits increased their breathing frequency. There was no clear change in the activity of six receptors in two nonparalysed bilaterally vagotomized rabbits. In no instance was a stretch receptor inhibited by ammonia.

We also tested the effect of carbon dioxide (CO_2) on thirteen pulmonary stretch receptors in six bilaterally vagotomized, paralysed rabbits. The rabbits were first hyperventilated until end-tidal PCO_2 was 14.6 ± 0.4 torr (mean and standard error). 8% CO_2 was then used to ventilate the animals, without change in pump stroke and frequency. Each of the thirteen receptors was inhibited, the percentage changes in mean impulse frequency (per pump cycle) being $-15.4 \pm 2.7\%$ ($P < 0.01$) for inflation and $-39.1 \pm 5.3\%$ ($P < 0.01$) for deflation. There was no measurable delay between change in discharge and change in end-tidal P_{CO_2}. Removal of the CO_2 produced recovery to control values.

Sixteen irritant receptors were studied in the vagotomized paralysed rabbits. 10-min exposure to SO_2 produced only small changes in activity. Six receptors increased their activity (fig. 3), the greatest increase being 57% in one fibre during the first minute of exposure. The increase in activity of the other five was in the range 20–30%. Two receptors were slightly inhibited while the remaining eight showed no clear change in activity.

Six irritant receptors studied in spontaneously breathing animals with the left vagus nerve intact had a mean spontaneous discharge of 3.8 ± 2.7 impulses $\cdot s^{-1}$. After exposing the rabbits to 200 ppm SO_2 for times ranging from 10-22 min the mean discharge was 7.3 ± 2.6 impulses $\cdot s^{-1}$.

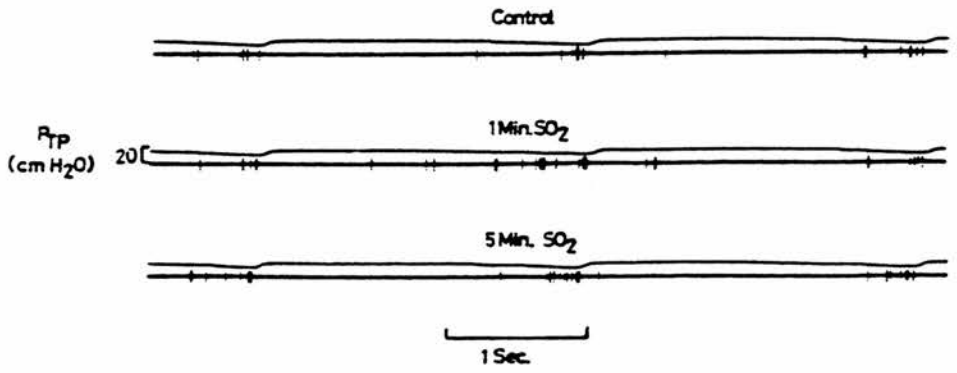

Fig. 3. Effect of 200 ppm SO_2 on the activity of a lung irritant receptor in a bilaterally vagotomized, paralysed rabbit. The traces show a single-fibre discharge recorded from the vagus nerve and transpulmonary pressure. Note that activity may be slightly increased in the second record, and is still present in the lowest record.

Pneumothorax and i.v. histamine (100 μg) always produced large increases in irritant receptor activity before and after exposure of the rabbits to SO_2. Both induction and removal of the pneumothorax stimulated the receptors. Similarly, the activity of three irritant receptors in two bilaterally vagotomized paralysed rabbits after exposure to SO_2 was markedly increased by addition of 2,200 ppm ammonia to their inspired air.

Breuer-Hering inflation reflex

Since SO_2 greatly diminished the activity of pulmonary stretch receptors, which mediate the Breuer-Hering reflex, we tested the strength of the reflex before and after SO_2 exposure.

Inflation of the lungs of ten rabbits with a positive pressure of 10 cm H_2O produced an average pause of 11.0 times the duration of the previous breath in the control animals (fig. 4). In the same animals after exposure to SO_2, inflation caused an average increase in cycle duration to only 1.2 times the previous breath. In four of the ten rabbits the ratio was reduced to unity, *i.e.* the Breuer-Hering reflex was completely abolished. At the end of 60 min after removal of the SO_2 the size of the Breuer-Hering reflex had been restored to its control value.

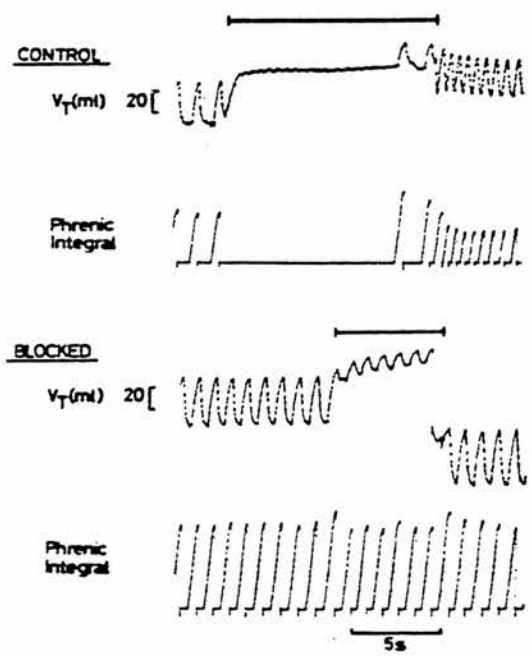

Fig. 4. Effect of pulmonary stretch receptor block by 10 min exposure to 200 ppm SO_2 on the inflation reflex. The traces are tidal volume and integrated phrenic discharge. The lungs were inflated by a positive pressure of 10 cm H_2O during the signal bars. The shift in base line in the lower volume trace was due to resetting the airflow integrator. Note the absence of the Breuer-Hering reflex after exposure to SO_2.

TABLE 2
Effect of intravenous histamine before and during stretch receptor block

	Control		Blocked	
	Before histamine	After histamine	Before histamine	After histamine
Inspiratory time t_I (s)	0.48 ± 0.12	0.43 ± 0.12	0.64 ± 0.10	0.72 ± 0.13
Expiratory time t_E (s)	0.94 ± 0.39	0.40 ± 0.27**	0.71 ± 0.17	0.38 ± 0.07**
Tidal volume V_T (ml)	21.5 ± 7.4	15.3 ± 5.1**	23.4 ± 5.0	18.9 ± 4.3**
Phrenic height H (mm)	43.6 ± 10.0	42.7 ± 9.0	39.2 ± 15.0	37.3 ± 20.0
Phrenic slope S (°)	41.6 ± 11.5	43.5 ± 12.0	35.5 ± 13.0	36.7 ± 14.0

Significance of differences from pre-histamine values, ** $P < 0.01$, by sign test: n = 15 for t_I, t_E and V_T; n = 8 for H and S.

Histamine

Since rabbits exposed to SO_2 had their Breuer-Hering reflex virtually abolished, we were able to use the preparation to test the action of other lung reflexes in the absence of pulmonary stretch receptor activity. After each test the Breuer-Hering reflex was assessed; only if it was absent was the result accepted as valid for the absence of pulmonary receptor discharge. We first describe the effect of histamine, which primarily stimulates lung irritant receptors (see Discussion).

In fifteen rabbits with stretch receptor activity intact, intravenous injections of histamine produced a small statistically insignificant decrease in t_I and a significant decrease in t_E and V_T (table 2). With stretch receptor activity inhibited by SO_2, histamine never reduced t_I and in many cases t_I slightly increased; the effect of histamine on t_E was not significantly different from the control result. The height and slope of the phrenic integral were not significantly changed by histamine in the presence or absence of stretch receptor activity. Change in end-expiratory volume was measured in 7 rabbits (see Discussion). The small significant ($P < 0.05$) increase ($+3.7 \pm 1.3$ ml) seen with histamine in controls became insignificant ($+8.5 \pm 3.5$ ml) after stretch receptor inhibition, the difference between the means also being statistically insignificant. After vagotomy histamine had no effect on pattern of breathing or on end-expiratory volume.

Phenyl diguanide

Phenyl diguanide, which primarily stimulates lung J-receptors (see Discussion), reduced t_E and V_T before and during stretch receptor block by SO_2 in fourteen rabbits (table 3). The effects in the two conditions were not significantly different. t_I was shortened by phenyl diguanide before the block, but during the block phenyl diguanide caused an insignificant increase in t_I. The effect of phenyl diguanide on end-expiratory volume was measured in six rabbits. Before stretch receptor block it increased by a mean of $+5.6 \pm 1.2$ ml ($P < 0.05$). After the block significantly

TABLE 3
Effect of intravenous phenyl diguanide (PDG) before and during stretch receptor block

	Control		Blocked	
	Before PDG	After PDG	Before PDG	After PDG
Inspiratory time t_I (s)	0.48 ± 0.14	0.37 ± 0.06**	0.60 ± 0.13	0.64 ± 0.14
Expiratory time t_E (s)	1.05 ± 0.56	0.27 ± 0.10**	0.62 ± 0.19	0.23 ± 0.10**
Tidal volume V_T (ml)	22.1 ± 7.4	14.2 ± 5.2**	27.0 ± 11.6	13.9 ± 4.0**
Phrenic height H (mm)	42.8 ± 10.0	34.5 ± 9.0**	43.5 ± 16.4	49.0 ± 16.0
Phrenic sope S (°)	44.0 ± 12.0	41.0 ± 11.0	37.1 ± 14.0	38.7 ± 13.0

Significance of differences from pre-PDG values, ** $P < 0.01$, by sign test; n = 14 for t_I, t_E and V_T, n = 7 for H and S.

($P < 0.05$) larger increases occurred in all animals (mean +17.0 ± 4.0 ml; $P < 0.01$). The height of the phrenic integral was significantly reduced by phenyl diguanide before stretch receptor block but not during block, and the slope of the phrenic integral was not significantly altered by phenyl diguanide in either condition. After vagotomy phenyl diguanide had no effect on pattern of breathing or on end-expiratory volume.

Carbon dioxide

CO_2 was added to the inspired air of two groups of rabbits. In the first group of twelve animals end-tidal P_{CO_2} was increased in two stages from about 34 torr first to about 51 and then to about 60 torr (group mean values). CO_2 was given in three conditions; controls, after exposure to SO_2, and after vagotomy (fig. 5). Figure 5 summarizes some of the results, and table 4 gives values for the air controls and the higher levels of CO_2. In the controls CO_2 increased frequency of breathing, almost entirely by a decrease in t_E, and also increased V_T. After exposure to SO_2, these rabbits responded to CO_2 with a greater increase in ventilation and frequency, the latter now being due

TABLE 4
Effect of breathing CO_2 before and after stretch receptor block and after vagotomy

	Control		SO_2-blocked		Vagotomized	
	Air	CO_2	Air	CO_2	Air	CO_2
CO_2 (torr)	38.3 ±1.7	60.2 ±1.4**	37.4 ±1.9	60.7 ±1.1**	37.4 ±1.9	61.5 ±1.1**
t_I (s)	0.46±0.03	0.42±0.03	1.07±0.14	0.55±0.05**†	1.77±0.40	0.98±0.13**†
t_E (s)	0.79±0.27	0.59±0.13*	0.55±0.09	0.49±0.08	1.17±0.20	1.36±0.20
V_T (ml)	16.3 ±1.70	23.2 ±1.36**	23.3 ±1.20	31.5 ±1.90**	29.4 ±2.38	39.7 ±3.47**†
F (br · min^{-1})	63.9 ±7.99	71.3 ±7.19*	42.6 ±4.13	61.4 ±5.28**†	26.9 ±4.08	30.4 ±2.89
$\dot{V}_T$ (l · min^{-1})	1.04±0.13	1.67±0.20**	1.01±0.12	1.96±0.25**†	0.89±0.15	1.32±0.17*

Significance of difference of response to CO_2 compared with air breathing, * $P < 0.05$, ** $P < 0.01$. Significance of difference of response to CO_2 in blocked and vagotomized conditions compared with control, † $P < 0.05$, †$P < 0.01$. CO_2 values are end-tidal.

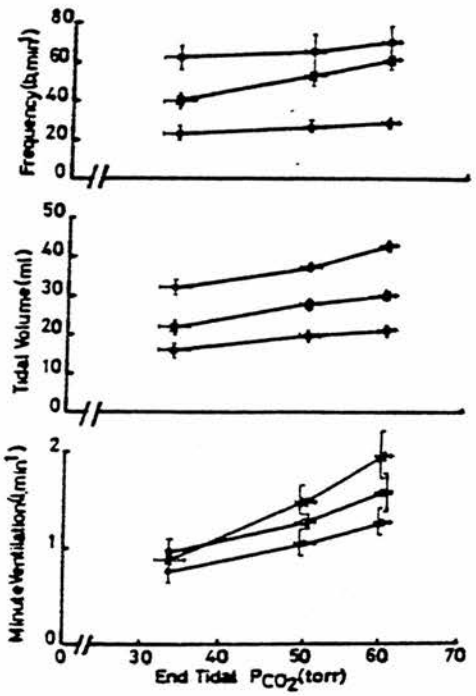

Fig. 5. Effect of increased end-tidal P_{CO_2} on, from above down, breathing frequency, tidal volume and minute ventilation in control (O), stretch receptor blocked (×) and vagotomized (●) rabbits. Vertical and horizontal lines are SEM.

to a decrease in tI. After vagotomy the increases in ventilation and frequency due to CO_2 were smaller, but there was still a significant increase in VT and a decrease in tI.

In a second group of seven animals for which phrenic activity was recorded, end-tidal CO_2 was increased from about 37 to about 51 torr (means). The ventilatory response of these animals was qualitatively the same as for the first group. The reduction in tI with CO_2 inhalation was consistently accompanied by an increase in the phrenic integral slope (27.6 ± 8.0^2 to 37.8 ± 13.0^2; $P < 0.05$). This effect persisted in the absence of stretch receptor activity (30.5 ± 14.5^2 to 37.5 ± 12.5^2; $P < 0.01$) and after vagotomy (35.3 ± 11.6^2 to 41.0 ± 13.8^2; $P < 0.01$).

Pneumothorax

The effect of pneumothorax was analysed mainly on the first breaths after induction and after removal of the pneumothorax, so that the effect of changes in blood gas tensions would be absent or minimal.

Before stretch receptor block induction of pneumothorax resulted in an increase in tI, this being greatest in the first breath (fig. 6, table 5). During stretch receptor block pneumothorax produced a far greater increase in tI than in the control. The decrease in tE during block was less than in the control and the changes in VT were

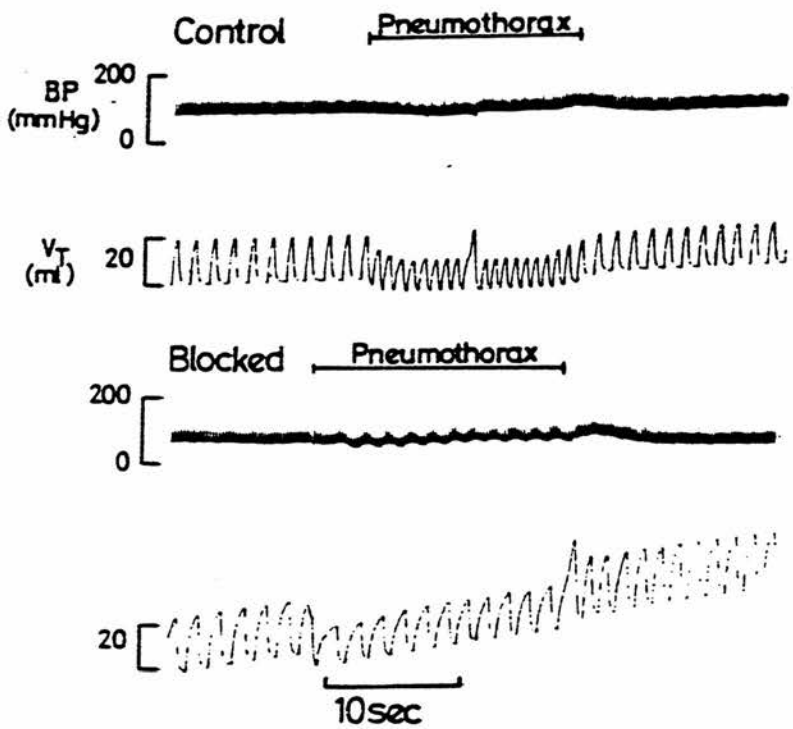

Fig. 6. Effect of pulmonary stretch receptor block by SO_2 on the changes in blood pressure and breathing produced by pneumothorax. A 40-ml pneumothorax was induced during the horizontal signal bars. The tidal volume record shows integrator drift. Note the prolongation of t_I on induction of the pneumothorax and the augmented breath on removal.

TABLE 5

Effect of pneumothorax before and after stretch receptor block on the first breath after pneumothorax

	Control		SO_2-blocked	
	Pre-pneumothorax	1st breath	Pre-pneumothorax	1st breath
t_I (s)	0.51 ± 0.07	0.65 ± 0.12*	0.83 ± 0.16	1.66 ± 0.57*+
t_E (s)	0.91 ± 0.21	0.32 ± 0.12*	0.80 ± 0.36	0.53 ± 0.25**+
V_T (ml)	19.2 ± 0.83	12.5 ± 2.50**	24.3 ± 3.2	15.8 ± 3.2**
H (mm)	35.3 ± 17.3	61.6 ± 16.5*	52.5 ± 17.5	81.7 ± 15.0*
S (°)	31.7 ± 9.8	39.3 ± 7.0	37.3 ± 8.4	38.8 ± 8.0

Significance of difference of response of 1st breath to pre-pneumothorax, * $P < 0.05$; ** $P < 0.01$. Significance of difference of response during receptor block compared with control, + $P < 0.05$, + $P < 0.01$.

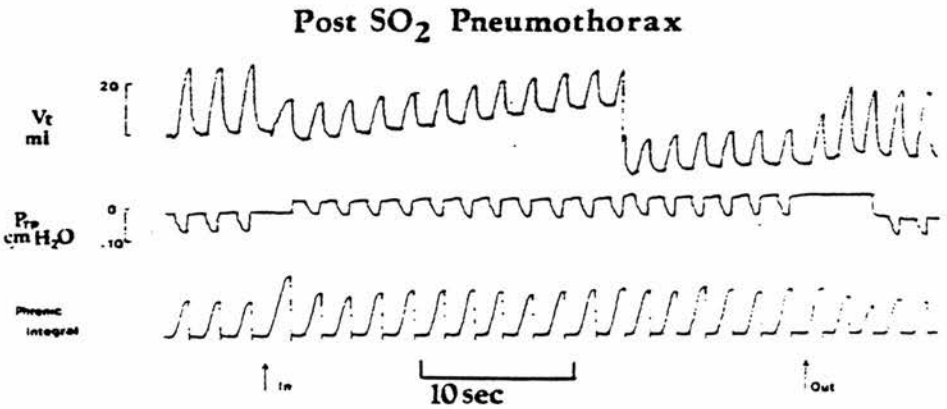

Fig. 7. Effect of pneumothorax in a rabbit after SO_2 on tidal volume (V_T), transpulmonary pressure (P_{TP}) and integrated phrenic electroneurogram. Arrows indicate induction and removal of pneumothorax. Tidal volume record shows integrator drift and resetting. The transpulmonary pressure record is interrupted during induction and removal of the pneumothorax. Note the increased phrenic height and inspiratory duration on induction of the pneumothorax.

not significantly different in the two conditions. Withdrawal of the pneumothorax nearly always reduced t_I in both the control and receptor-blocked states (fig. 7), but occasionally an augmented breath was seen (fig. 6). t_E on the other hand was increased on removal of the pneumothorax in the control state but was reduced more in the receptor-blocked animals. The changes in end-expiratory volume produced by pneumothorax were smaller than the volume of the pneumothorax (figs. 6 and 7). The volume of injected air produced an outward movement of the chest wall as well as a reduction in lung volume. Since the chest wall of rabbits is 50% more compliant than their lungs (Crosfill and Widdicombe, 1961), lung collapse was the minor effect. There may also have been compensatory changes in respiratory muscle activity which would tend to maintain end-expiratory volume. Vagotomy abolished the changes in t_I and t_E produced by pneumothorax.

Histology

Light microscopy revealed no damage to the lungs, bronchi or trachea in any of the five animals exposed to SO_2 (for 1–5 periods of 10 min during 4–5 hr) and studied histologically. There was no visible stripping of the ciliary layer due to SO_2, and no alveolar or mucosal oedema or vascular congestion. The goblet cells in the trachea and left main bronchus still contained inclusion globules of glycoprotein, stained histochemically.

Blood gases and pH

pH, P_{O_2} and P_{CO_2} were measured in samples of arterial blood taken from the femoral catheter of five spontaneously breathing rabbits (six experiments) before ($pH = 7.37 \pm 0.027$; $P_{O_2} = 91.3 \pm 10.50$ torr; $P_{CO_2} = 49.2 \pm 8.0$ torr), and immedia-

tely after (pH = 7.38 ± 0.034; P_{O_2} = 83.6 ± 9.76 torr; P_{CO_2} = 43.1 ± 3.78 torr) 10 min exposure to SO_2. The only significant change was the decrease in P_{CO_2} (−6.0 ± 0.82 torr; $P < 0.01$), although this was not reflected in end-tidal CO_2 values in a different group of rabbits (table 4). Possibly the SO_2 caused an increase in ventilation/perfusion imbalance with mild hypoxia and hyperventilation.

Augmented breaths

Augmented breaths are typified by increased VT, a double inspiratory peak on the airflow record and a subsequent transient increase in FRC. They occurred in the control and receptor-blocked states but never after vagotomy. Augmented breaths occurred most frequently during CO_2 inhalation and during the bronchoconstriction due to SO_2 inhalation in many rabbits. In the latter condition the augmented breaths usually occurred when compliance was reduced and always resulted in an increase in compliance.

Discussion

We have observed that inhalation of 200 ppm SO_2 by rabbits abolished activity in pulmonary stretch receptors. The mechanism of this inhibition has not yet been clearly elucidated, but from our histological findings it seems unlikely to be due to frank damage to the airways. This view is supported by the fact that both receptors and the Breuer-Hering reflex recovered in 20–60 min and it was possible to repeat the procedure several times without loss of its effectiveness; in our experience the only limit has been the duration of an experiment on an anaesthetized rabbit (4–8 hr). It is now well established that carbon dioxide (CO_2) inhibits pulmonary stretch receptors (Bartlett and Sant'Ambrogio, 1976; Bradley *et al.*, 1976), and the inhibitory action of CO_2 may be due to a change in pH in the receptors' environment. As both CO_2 and SO_2 are acidic gases it might be expected that both these gases would decrease pH in the receptors' environment. The alkaline gas we tried, ammonia, which would *increase* tissue pH, usually stimulated stretch receptors and never caused inhibition. Boushey *et al.* (1974) have described responses of laryngeal epithelial receptors, which were inhibited by CO_2; however, these were not affected by 100 ppm SO_2. In our experiments any changes in pH must have been local to the lung receptors, since administration of SO_2 caused no significant change in blood pH; changes in blood (as compared with airway) P_{CO_2} do not influence the activity of stretch receptors in dogs (Bartlett and Sant'Ambrogio, 1976; Bradley *et al.*, 1976). The different patterns with which the stretch receptors silenced may have reflected their different sites within the tissues and the rate of increase in SO_2 concentration around them. The more powerful action of SO_2, compared with CO_2, in inhibiting stretch receptors may be because SO_2 is converted to sulphate (SO_4^{2-}) ions which are relatively nondiffusible compared with CO_2, but this speculation has not been tested by pH measurements close to the receptors.

Whatever was the precise cause of stretch receptor inhibition the changes in

pattern of breathing and reflexes from the lungs were well defined and we have attempted to explain them in terms of the receptor systems known to exist. Both the results with fibre recording and those with reflexes strongly suggest that, in the conditions of our experiments, SO_2 blocks the activity of pulmonary stretch receptors and abolishes the inflation reflex, while leaving the lung irritant and J-receptors and their reflexes intact. We cannot say definitely whether exposure to SO_2 changes the sensitivity of lung irritant and J-receptors, since the spontaneous activity of irritant receptors is so variable that statistical analysis does not reach significant values in our experiments, and we have not directly measured J-receptor activity. We can only say that irritant receptors were active after exposure to SO_2, and that irritant and J-receptor reflexes could still be elicited. SO_2-differential block is therefore highly specific, and has advantages over other methods. Vagal cooling (Dawes *et al.*, 1951; Widdicombe, 1967; Karczewski and Widdicombe, 1969) does not readily separate inflation and lung irritant reflexes, since both are mediated by myelinated vagal fibres, and may be distorted by impulse frequency-dependent effects (Paintal, 1965). Anodal block (Guz and Trenchard, 1971) does not separate inflation and irritant reflexes (Trenchard and Widdicombe, 1973), although it is useful for isolating the J-receptor reflex conducted by nonmyelinated fibres. Inhalation of local anaesthetic aerosols (Jain *et al.*, 1973; Cross *et al.*, 1976) has not been adequately tested on irritant reflexes or by recording activity in afferent fibres from irritant receptors, but seems to lack specificity and reproducibility. One aspect of the SO_2-method is that it does not produce a complete block of the inflation reflex in cats (our observations) or dogs (D. Trenchard, personal communication). However, in preliminary experiments we have found the method to work in rats.

SO_2-exposure increased t_I and V_T, changes consistent with the analysis of the mechanism of the inflation reflex by Clark and Euler (1972) for the cat. The reflex has a volume 'threshold' which when reached terminates inspiration, and this threshold may be dependent on timing during inspiration and other influences including CO_2 tension. As lung volume is monitored by stretch receptors this system depends on vagal integrity for its function. The further increase in t_I and V_T produced by vagotomy in the presence of stretch receptor block by SO_2 in our study has been observed with other forms of vagal block (Karczewski and Widdicombe, 1969; Nadel *et al.*, 1973; Phillipson *et al.*, 1973; Guz and Trenchard, 1971). The observation suggests the existence of a vagally-dependent control of t_I and V_T by a pathway other than that for the inflation reflex. This pathway is unlikely to be the few pulmonary stretch receptors not completely inhibited by SO_2, since they were shown to be ineffective in causing an inflation reflex response. It is most likely that irritant or J-receptors may be controlling t_I and V_T; stimulation of these endings is thought to decrease t_I and V_T with no change in 'phrenic slope' (Winning and Widdicombe, 1976), so abolition of their activity should increase t_I and V_T.

When stretch receptors were blocked in our experiments t_E was decreased, but additional vagotomy caused a large increase in t_E. The Breuer-Hering inflation reflex, mediated by pulmonary stretch receptors, essentially includes a prolongation

of t_E. Clark and Euler (1972) and Knox (1973) have shown that lung inflation in the expiratory phase of cats prolongs t_E. Bartoli *et al.* (1973) have confirmed this in dogs. The tonic activity of stretch receptors, 60% of which are active at end-expiratory volumes (Paintal, 1966), may be responsible for this control of t_E. The removal of this activity would explain the decrease in t_E seen with stretch receptor block. The increase in t_E seen with vagotomy during stretch receptor block suggests that activity of irritant or J-receptors may be shortening t_E (see below). This hypothesis may explain the paradox that, if one assumes that the inflation reflex is the only important vagal influence on normal pattern of breathing, vagotomy would be expected to shorten t_E whereas in practice it is lengthened. If our hypothesis is right, it also follows that, in our experimental conditions in the rabbit, the t_E-shortening effect of irritant and/or J-receptors is more powerful than the t_E-lengthening effect of stretch receptors.

The increase in breathing frequency brought about by carbon dioxide inhalation in control conditions was largely due to reduction in t_E, but also mainly due to a reduction in t_I in SO_2-blocked and vagotomized rabbits; Gautier (1973) reported similar changes in rabbits with CO_2 breathing. Bilateral vagotomy did not abolish the increase in frequency due to carbon dioxide in all animals, supporting the observation by Widdicombe and Winning (1974) that intact vagal circuits are not always essential for this effect.

The increase in minute ventilation due to carbon dioxide was greater in the stretch receptor-blocked than in the control or vagotomized condition in our first group of rabbits; this result supports the observation by Nadel *et al.* (1973) and Phillipson *et al.* (1973) that, with the inflation reflex of conscious dogs blocked by cooling but with the irritant reflex response to histamine still intact, there was a potentiation of the ventilatory response to carbon dioxide. This may have been generated by the increased irritant receptor activity which Sellick and Widdicombe (1969) demonstrated in CO_2-hyperpnoea, and is consistent with the small decrease of arterial P_{CO_2} caused by SO_2-exposure.

The stimulus of inhaled carbon dioxide was unique in producing a large change in the slope of the phrenic integral. This change persisted after bilateral vagotomy and could be due to an action of CO_2 on medullary chemoreceptors.

Although stretch receptor block did not abolish the respiratory responses to intravenous phenyl diguanide (suggesting that stretch receptors are not primarily involved in this reflex) the responses were somewhat modified. The largest difference was in the change in t_I. The reduction in t_I in the controls was abolished or converted to a small increase during stretch receptor block. One of the effects of phenyl diguanide was to increase FRC and this would tend to terminate inspiration earlier by increasing stretch receptor activity. In the absence of such activity changes in FRC would be ineffective in changing t_I. If phenyl diguanide is assumed to act only on lung J-receptors, our results suggest that the primary actions of the receptors in the rabbit are to decrease t_E and V_T and to increase FRC, with little effect on t_I or phrenic slope.

Recordings of nerve impulses from epithelial irritant receptors support the observation of Mills *et al.* (1969) that the receptors are stimulated by intravenous histamine. This observation, and the fact that there are almost identical ventilatory responses to histamine before and during stretch receptor block, suggest that irritant receptors may have a primary role in the reflex responses to histamine, but do not eliminate the involvement of other types of receptor in intact animals. Histamine sensitizes pulmonary stretch receptors (Widdicombe, 1961) and this may have played a part in the controls. If histamine is assumed to act only on lung irritant receptors, our results suggest that these receptors have an action very similar to that of J-receptors: decreases in t_E and V_T, with little effect on t_I and phrenic slope. Karczewski (1975) also found that for rabbits histamine shortened t_E with little change in t_I. However, histamine was far less effective than phenyl diguanide in increasing FRC in our study. Our results with phenyl diguanide and histamine confirm, in general, those of Winning and Widdicombe (1976) with cats. In that paper is discussed the specificity of action of the two drugs which is probably far from perfect. However, since both cause very similar effects (with the exception of the FRC changes) the conclusions about the reflex actions of irritant and J-receptors are probably valid.

The induction of pneumothorax in experimental animals may modify the activity of all three types of pulmonary vagal receptors. Stretch receptor activity is diminished (Knowlton and Larrabee, 1946; Luck, 1970), and irritant receptor activity is increased (Homberger, 1968; Sellick and Widdicombe, 1969). However, J-receptors are only slightly stimulated and then only in extreme degrees of lung collapse (Paintal, 1973). The most striking difference between the response to pneumothorax before and during stretch receptor block in our experiments was the prolongation of t_I in the first breath during block. The absence of such an effect after bilateral vagotomy indicates a reflex vagal mechanism. As stretch receptors were inactivated irritant receptors are the most likely source of this effect. In control conditions, pneumothorax shortened t_E, and the size of this effect was considerably less during stretch receptor block. If J-receptors were not stimulated by the pneumothorax, then during stretch receptor block irritant receptors must be responsible for decreasing t_E; and with intact vagi both stimulation of irritant receptors and inhibition of stretch receptors cause the even greater shortening of t_E. Removal of the pneumothorax, known to stimulate irritant receptors, during stretch receptor block nearly always decreased both t_I and t_E. Thus, with stretch receptor block and therefore presumably via irritant receptors, both induction and removal of pneumothorax shorten t_E but the former increases and the latter decreases t_I. The failure of removal of pneumothorax to prolong t_I could be because the inspiratory augmenting reflex shows refractoriness (Reynolds, 1962); the rare occasions when removal of the pneumothorax caused an augmented breath (fig. 6) may have been because induction of the pneumothorax was not a sufficiently strong stimulus to make the reflex refractory.

Collating the results in this paper leads to the following conclusions, based on the assumptions (1) that SO_2-exposure blocks the activity of pulmonary stretch recep-

tors, and (2) that during SO_2-exposure lung irritant receptors are the only ones spontaneously active and stimulated by carbon dioxide, pneumothorax and histamine. For t_E, the results with vagotomy during SO_2 exposure, histamine and induction and removal of pneumothorax all support the view that irritant receptors shorten t_E. For t_I, the position is more complex: the results with vagotomy during SO_2 exposure, removal of pneumothorax and possibly CO_2-breathing all suggest that irritant receptors shorten t_I; however, the results with induction of pneumothorax indicate the opposite, and those with histamine show only a small effect on t_I. Furthermore, evidence has been published that irritant receptors are responsible for augmented breaths (Sellick and Widdicombe, 1970; Glogowska *et al.*, 1972) and our results in this paper are consistent with this view. Research is in progress to resolve this problem, based on the hypothesis that the reflex response of t_I to irritant receptor activity depends on the pattern and timing of the afferent discharge, and on the possibility that the inspiratory augmenting reflex may show refractoriness. For ventilation, the results with blood gases and SO_2-exposure, and those with CO_2 breathing, are consistent with the view that pulmonary stretch receptors inhibit alveolar ventilation (Richardson and Widdicombe, 1969) and lung irritant receptors augment it.

Our results support the view that J-receptors shorten t_E, although Winning and Widdicombe (1976) found that they also shortened t_I in the cat. The J-receptors may also have contributed to the responses ascribed above to irritant receptors. Although J-receptors show little response to volume changes of the lungs (Paintal, 1973; Sellick and Widdicombe, 1970) and have no tonic reflex effect on breathing in rabbits with healthy lungs (Guz and Trenchard, 1971), they do respond to intravenous histamine (Paintal, 1974); furthermore it is not known if SO_2-exposure may stimulate or sensitize J-receptors.

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Patterns of accelerated breathing provoked by lung irritant receptor activity.

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Patterns of accelerated breathing provoked by lung irritant receptor activity

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The fact that stimuli which increase lung irritant receptor activity also accelerate breathing has been well established (Mills, Sellick & Widdicombe, 1969; Koller & Ferrer, 1973). Breathing frequency may be accelerated by a reduction in inspiratory duration (t_I), a reduction in expiratory duration (t_E) or a combination of both of these; this complexity has rendered difficult the interpretation of changes in pattern in terms of lung receptor activity.

We restrict our observations in this communication to the effects of histamine acid phosphate given by aerosol inhalation (2%, $n = 25$) and intravenously (100–900 μg, $n = 9$) to rabbits ($n = 15$) anaesthetized with pentobarbitone sodium (40 mg/kg). The duration of inspiration and expiration were measured from the discharge in a root of the right phrenic nerve. Under these conditions acceleration of breathing usually took one of two forms. In the first ($n = 6$) both t_I and t_E shortened gradually but not necessarily in the same proportion. The changes in t_I did not begin until one or two breaths after t_E had begun to shorten. In the second type ($n = 20$) t_E shortened gradually but t_I remained unchanged until an augmented breath occurred, when t_I immediately shortened and underwent further gradual shortening before recovery. We term these two patterns continuous and discontinuous acceleration respectively. In a further eight tests the pattern was not clear or was obscured by coughs.

Reynolds (1962) described patterns of breathing associated with augmented breaths. In a previous communication (Davies, 1976) we have demonstrated how an augmented breath can influence t_I for some time after it has occurred. We therefore supposed that the continuous acceleration we have described was the result of a spontaneously occurring augmented breath exerting its influence over the period of irritant receptor activity which produced the acceleration.

To test this hypothesis we provoked augmented breaths ($n = 12$) by a method already described (Davies & Roumy, 1978) in which brief (100 msec) pulses of inflation or deflation were applied to the lungs, and then we immediately administered histamine by aerosol or by injection. Under these conditions acceleration was continuous ($n = 12$).

In a previous communication (Davies, Roumy, Widdicombe & Wise, 1977) we demonstrated the association between lung irritant receptor activity and augmented breaths. We suggest that our present results demonstrate a degree of independence of t_I and t_E, and that the pattern of accelerated breathing seen under the conditions of our experiments is determined by the proximity of an augmented breath, or more probably by the level of irritant receptor activity associated with the augmented breath which may link the durations of t_I and t_E.

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Publication 20.

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Effect of repeated exposures to high concentrations of sulphur dioxide on respiratory reflexes in rabbits.

Bull. Europ. Physiopath. Resp. 14, 41-52.

EFFECT OF REPEATED EXPOSURES TO HIGH CONCENTRATIONS OF SULPHUR DIOXIDE ON RESPIRATORY REFLEXES IN RABBITS

EFFET D'EXPOSITIONS RÉPÉTÉES A DES CONCENTRATIONS ÉLEVÉES D'ANHYDRIDE SULFUREUX SUR LES RÉFLEXES RESPIRATOIRES DU LAPIN

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ABSTRACT

Rabbits were exposed to sulphur dioxide, either 300 ppm for 3 h or 150 ppm for twelve 3 h periods. After exposure we measured (1) lung mechanics, (2) Breuer-Hering inflation reflex, (3) breathing responses to inhaled carbon dioxide and ammonia, pneumothorax and intravenous injections of histamine and phenyl diguanide, and (4) activity of pulmonary stretch and lung irritant receptors by single-fibre recording. The results indicate that chronic exposure to SO_2 inhibits pulmonary stretch receptor activity and its reflex effects, and raises the question whether this mechanism may play a part in the control of breathing in human patients with chronic bronchitis.

Breuer-Hering reflex ; chronic bronchitis ; pulmonary stretch receptor ; sulphur dioxide.

INTRODUCTION

The acute effects of sulphur dioxide (SO_2) have been extensively studied on specific aspects of respiratory physiology such as breathing, bronchial calibre and mucus transport (*e.g.* [6, 7, 8, 24, 27]). The relationship between inhalation of sulphur dioxide and chronic respiratory disease, and the use of sulphur dioxide to produce experimental chronic bronchitis, have also been widely reported [1, 4, 9, 13, 25], but the studies have been generally limited to histopathology and lung mechanics. However, chronic bronchitis due to inhalation of irritant gases might

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be expected to change the behaviour of nervous receptors in the airways, and thus the nervous control of breathing. In this paper we describe experiments designed to test the effects of chronic exposure of rabbits to SO_2 on the lung reflex control of breathing, in the hope that results might be relevant to chronic bronchitis. We have also studied changes during a recovery period after exposure.

METHODS

Experiments were performed on 50 New Zealand white adult rabbits of either sex, body weight 3 to 4.2 kg. In initial experiments eight rabbits received a single 3 h exposure to 300 ppm SO_2 and were compared with a group of 18 control animals. These groups will be called *single high-dose exposure*. The remaining 24 rabbits were divided into four equal groups of six ; three groups were exposed to 150 ppm SO_2 for twelve 3 h periods on alternate days over four weeks in a Perspex chamber 65 × 55 × 34 cm. The last group of six rabbits were controls submitted to the same exposure regime but without SO_2 added to their environment. These groups will be called *multiple low-dose exposure*. The single high-dose exposure rabbits were investigated three days after exposure. The multiple low-dose exposure rabbits were investigated one, two or seven days (three groups of eight rabbits each) after their final exposure to SO_2.

The SO_2 mixture was made by injecting pure SO_2 (B.O.C., Special Gases) at a measured fixed rate into a measured stream of air blown into the chamber. A diffuser ensured that the gas was uniformly distributed in the chamber. The gas escaped to the atmosphere through a water washing tower which removed all traces of SO_2. The concentration of SO_2 in the chamber was measured at the beginning of each exposure with Drager tubes and the necessary adjustment was made to the flow of SO_2 to achieve the desired concentration. The concentration of SO_2 was checked hourly throughout each exposure, with Drager tubes.

Rabbits in each group were anaesthetized with 32 mg/kg of sodium pentobarbitone (Nembutal, Abbott) intravenously. Supplementary doses were given when appropriate to maintain surgical anaesthesia. Surgical preparation consisted of tracheal cannulation, the insertion of polyethylene catheters into a femoral artery and vein and the exposure of both cervical vagi.

Blood pressure was recorded from the arterial catheter using a strain-gauge manometer (C.E.C.). Airflow was measured from a Fleisch pneumotachograph head in series with the tracheal cannula. The pressure difference across the head was measured with a strain-gauge differential manometer (Statham) and integrated electronically to give volume changes. Transpulmonary pressure was measured after inserting a cannula into the right intra-pleural space. The cannula was connected to one side of a capacitance micro-manometer (Mercury Instruments), the other side of the manometer being connected to the tracheal cannula on the tracheal side of the pneumotachograph head. Airway carbon dioxide was measured with a infrared absorption meter (Beckman Spinco L.B.1) which sampled gas at 300 ml · min^{-1} from the external side of the pneumotachograph head.

To test various reflex and chemical factors controlling breathing, each animal was subjected to one or more of the following experimental procedures :

(1) To test the response to carbon dioxide, the rabbits was made to breathe sequentially air containing 4, 6 and 8 % carbon dioxide for 3 min ;

(2) To test the Breuer-Hering inflation reflex, the lungs were inflated with positive pressures of 5, 10 and 15 cm H_2O and the durations of the resulting inhibitions of inspiration were noted ;

(3) To test the response to pneumothorax, which would inhibit pulmonary stretch receptors and stimulate lung irritant receptors, 40 ml of air was introduced quickly through the pleural catheter and removed approximately ten breaths later ;

(4) To test the response to stimulation of lung irritant and cough receptors, the rabbit breathed from a passing stream of air flowing at 5 l · min^{-1} into which 40 ml · min^{-1} of air saturated with ammonia vapour was slowly injected (giving an ammonia concentration of 2200 ppm) ;

(5) To test the responses to chemical stimulation of lung irritant and J-receptors, 100 μg doses of histamine acid phosphate and phenyl diguanide dihydrochloride dissolved in 0.9 % saline were injected intravenously (doses as salts per animal).

After these tests the nerve impulse traffic from the lungs was studied. Each rabbit was paralysed with an intravenous injection of 15 $mg \cdot kg^{-1}$ of gallamine triethiodide (Flaxedil, May and Baker) and ventilated by a Palmer Pump at a rate to maintain its end-tidal CO_2 % at pre-paralysis level. Further doses of gallamine were given when necessary. Paralysed animals received hourly supplementary doses of anaesthetic. Action potentials were recorded from the distal end of the cut right vagus nerve. The nerve was laid in a copper tray containing mineral oil, and 'single-fibre' preparations were made. Platinum electrodes and Tektronix 122 pre-amplifiers were used. The amplified action potentials were fed into an instantaneous frequency meter. During recording of vagal action potentials from pulmonary stretch receptors, the following tests were performed: (1) the rabbits which had received the single high concentration of SO_2 were all ventilated with a stroke volume of 26 ml and a rate of 1 Hz, and the instantaneous frequency of discharge of individual stretch receptors was noted at 4 ml intervals of stroke volume; and (2) the lungs were inflated by constant positive pressures of 5, 10 and 15 cm of water in sequence. Lung irritant receptor activity was tested by large lung inflations and deflations, injections of histamine and insufflations of ammonia [16, 17], as already described above for reflex studies.

In all experiments records of blood pressure, airflow, tidal volume, transpulmonary pressure, airway CO_2 % and instantaneous action potential frequency were recorded on ultraviolet sensitive paper (Honeywell U.V. 31), and at the same time stored, together with a spoken commentary, on magnetic tape (Ampex S.P. 300). Airflow and transpulmonary pressure, from which a variable fraction of the volume signal was subtracted, were displayed on the X and Y axes of an oscilloscope so that total lung resistance and compliance could be calculated by the subtractor method of MEAD and WHITTENBERGER [15] as modified by NADEL and WIDDICOMBE [18].

RESULTS

The rabbits tolerated the exposure to SO_2 without obvious discomfort or stress. After exposure most rabbits had nasal mucus discharges, but these disappeared after one day. Three days after exposure, all but one rabbit appeared healthy with no evidence of nasal discharge or laboured breathing.

Lung mechanics

Single high-dose exposure. These animals had a higher total lung resistance (56.3 ± 9.8 $cmH_2O \cdot l^{-1} \cdot s$; mean and standard error, $n = 8$) than a group of control animals (25.6 ± 2.7 $cmH_2O \cdot l^{-1} \cdot s$; $n = 18$, $p < 0.01$); measurements were made three days after exposure. There was no significant difference in dynamic lung compliance (8.1 ± 0.62 $ml \cdot cmH_2O^{-1}$, control; 7.1 ± 0.76 $ml \cdot cmH_2O^{-1}$, exposed).

Multiple low-dose exposure. The pooled data for 1, 2 and 7 days exposed animals showed no significant difference in lung mechanics from controls (total lung resistance 31.8 ± 1.4 $cmH_2O \cdot l^{-1} \cdot s$, control; 30.0 ± 4.4 $cmH_2O \cdot l^{-1} \cdot s$, exposed; compliance 5.7 ± 1.6 $ml \cdot cmH_2O^{-1}$, control; 9.4 ± 3.0 $ml \cdot cmH_2O^{-1}$, exposed; $n = 6$). For both groups, upper airways resistance was not measured since the trachea was cannulated, but the exposed animals appeared to produce more nasal mucus than the controls which were not exposed.

Breathing frequency

Single high-dose exposure. During exposure breathing frequency slowed, and after exposure frequency recovered slowly to control values over a period of about 1 h.

Multiple low-dose exposure. Both exposed and control animals were drawn from the same population with a breathing frequency of 121 ± 3.8 breaths · min^{-1} before exposure and without anaesthesia. During exposure to SO_2 the breathing frequency of the experimental rabbits was 110 ± 6.8 breaths · min^{-1} and that of the control rabbits exposed to air was 123 ± 8.4. At 1 h after exposure the breathing frequency of the controls was 120 ± 4.9 and the exposed animals 89.31 ± 7.4 breaths · min^{-1} ($n = 6$, $p < 0.01$).

Breuer-Hering inflation reflex

Multiple low-dose exposure. The inflation reflex was assessed as the ratio of the apnoeic pause caused by inflation of the lungs to the mean preceding cycle duration. With positive-pressure inflations of 15 cmH_2O the ratio was significantly less in all the repeatedly exposed animals (14.3 ± 2.5, $n = 12$, compared to 20.4 ± 1.8, $n = 6$; $p < 0.01$) (fig. 1). At lower inflation pressures the diffe-

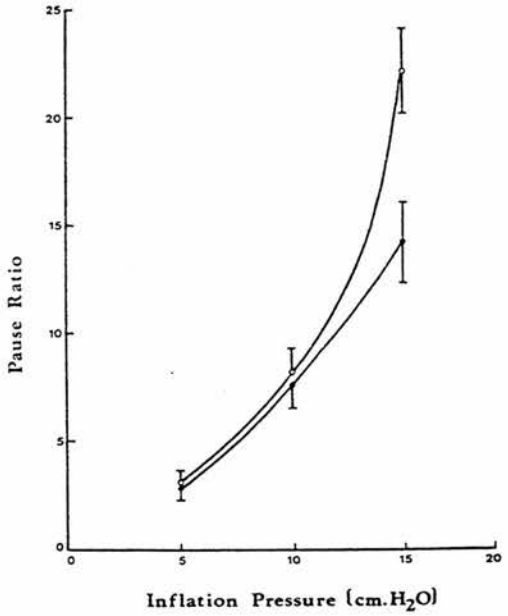

Fig. 1. — Effect of multiple low-dose exposure to SO_2 on the Breuer-Hering reflex. Ordinate, the inspiratory inhibitory ratio due to inflation of the lungs; abscissa, the inflation pressure used to elicit the reflex. Open circles, controls; filled circles, after exposure to SO_2. In this, and subsequent figures, the values are means and standard errors. At the highest inflation pressure (15 cmH_2O) exposure to SO_2 caused a significant ($p < 0.01$) reduction in the strength of the Breuer-Hering reflex.

rences were not statistically significant. There was no clear trend in the strength of the reflex from day 1 to 7 after the last exposure.

Responses to carbon dioxide, ammonia and pneumothorax

Multiple low-dose exposure. The breathing frequency and minute volume responses to inhaled 4, 6 and 8 % CO_2 were enhanced one day (n = 4) after the last exposure (fig. 2), as were the responses to inhaled ammonia (fig. 3) and

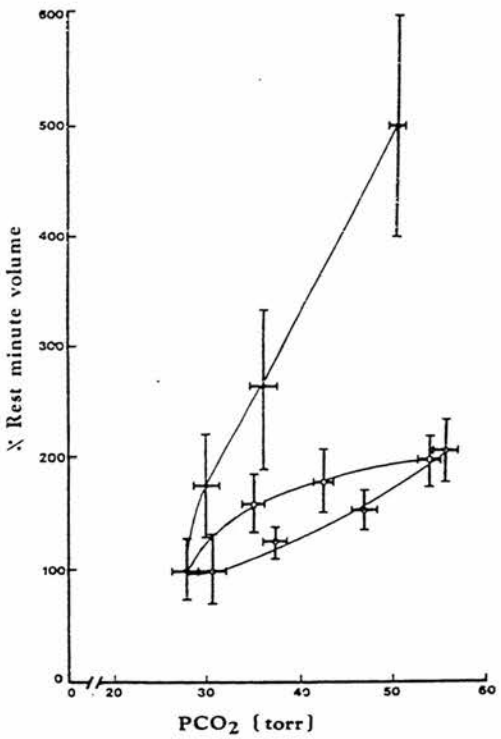

Fig. 2. — Effect of multiple low-dose exposure to SO_2 on the response to CO_2. Ordinate, minute volume as a percentage of the control value breathing air ; abscissa, end-tidal P_{CO_2}. Open circles, controls ; filled circles, one day after last exposure to SO_2 ; squares, seven days after last exposure to SO_2. There was a significant ($p < 0.01$) increase in the response to CO_2 one day after the last exposure to SO_2.

to pneumothorax. For example, inhalation of 8 % CO_2 increased breathing frequency by 20 %, 105 %, 50 % and 22 % for controls and 1, 2 and 7 days after exposure respectively. These enhancements were transitory and the responses of animals last exposed to SO_2 seven days (n = 4) before were not significantly different from those of the unexposed controls (n = 6).

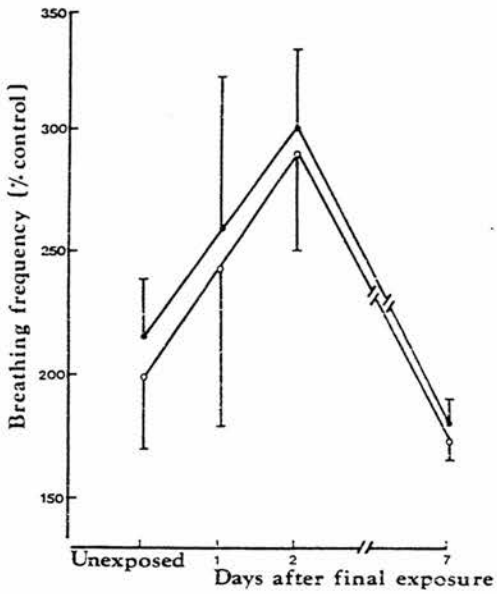

Fig. 3. — Effect of multiple low-dose exposure to SO_2 on the breathing frequency responses to inhaled ammonia and to pneumothorax. Ordinate, breathing frequency as a percentage of control ; abscissa, days after last exposure to SO_2. Open circles, pneumothorax ; closed circles, ammonia. The responses were enhanced two days after exposure but had recovered by seven days.

Responses to histamine and phenyl diguanide

Multiple low-dose exposure. The breathing frequency responses of the exposed animals to histamine and phenyl diguanide, which stimulate lung irritant and J-receptors, were not appreciably different from those of the unexposed animals.

Lung receptor activity

Single high-dose exposure. The rate of discharge of 26 pulmonary stretch receptors showed a plateau at lung volumes greater than 18 ml, whereas the controls (30 fibres) did not (fig. 4). The ratio of fibres from pulmonary stretch receptors to those from lung irritant receptors in the exposed animals was 4.3 : 1, whereas in the controls the ratio was 10 : 1 ($p < 0.05$, by χ^2 test).

Multiple low-dose exposure. Thirteen receptors from these animals at 1, 2 or 7 days did not show any significant difference in discharge frequency from seventeen receptors in the unexposed animals at any level of inflation. The ratio of stretch to irritant receptors found in all the exposed animals was 8 : 1 (2 out of 17) compared with the control ratio of 10 to 1 (1 out of 13 ; difference not significant, χ^2 test). In addition four fibres were found in the exposed animals that came from receptors with a low-frequency irregular discharge only slightly modu-

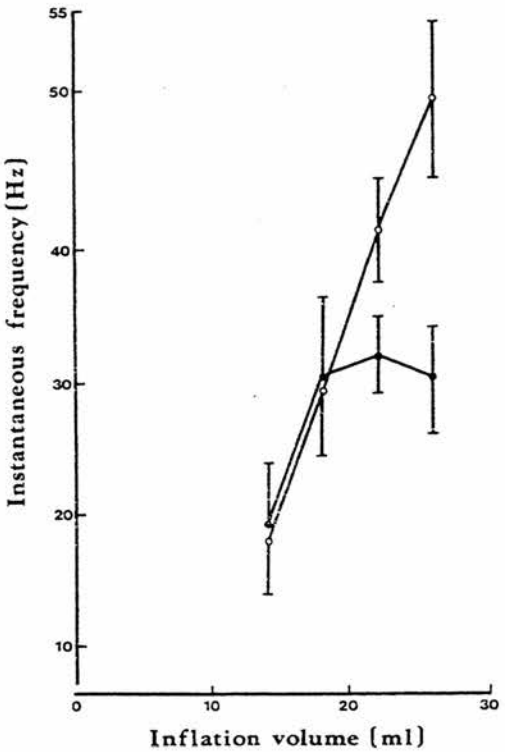

Fig. 4. — Effect of single high-dose exposure to SO_2 on the activity of pulmonary stretch receptors. Ordinate, frequency of discharge ; abscissa, inflation volume. Open circles, controls ; filled circles, after exposure to SO_2. At larger volume lung inflations the response of the pulmonary stretch receptors was significantly reduced by exposure to SO_2.

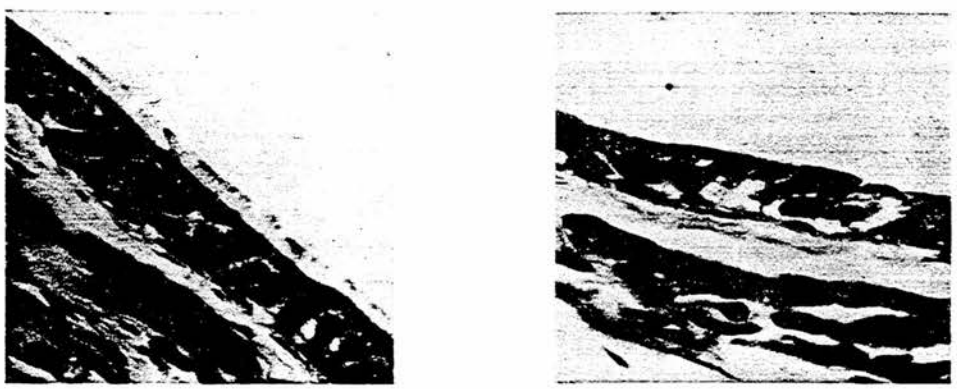

Fig. 5. — Histological appearance of the tracheal wall before (A) and after (B) a single high-dose exposure to SO_2. Haemotoxylin and eosin. Exposure to SO_2 damages the ciliary layer but does not destroy the epithelium.

lated by inflation of the lungs. Similar fibres were not found in the controls and may have arisen from damaged receptors.

Histology

Sections taken from the trachea and large bronchi of all the exposed rabbits showed absence of the ciliary layer but the epithelium was otherwise intact and its cells had normal appearances with light microscopy (fig. 5).

DISCUSSION

The aim of these experiments was to produce a mild degree of chronic bronchitis and to see if nervous control of breathing from lung reflexes was changed. Lung receptor behaviour is altered by acute exposure to SO_2 [7, 8, 28] and we postulated that chronic exposure might cause similar long term effects. The initial experiments with single high-dose exposure indicated that this postulate was justified, so the larger series with multiple low-dose exposure was carried out.

Table I summarizes qualitatively the results already described. Nearly all the results are consistent with the hypothesis that exposure to SO_2 inhibits the activity of pulmonary stretch receptors. JAY *et al.* [10] have reported, in abstract, a similar conclusion.

TABLE I

Summary of the responses to two patterns of exposure to SO_2

	Multiple low-dose exposure	Single high-dose exposure
Total lung resistance	=	↑
Lung compliance	(↑)	0
Breathing frequency during exposure	↓	↓
Breathing frequency after exposure	↓	=
Pulmonary stretch receptor activity	↓	↓
Breuer-Hering inflation reflex	↓	
Frequency response to CO_2, etc.	↑	

(1) Direct measurement of stretch receptor activity showed either that it was depressed at larger lung volumes, or that the ratio of stretch receptors to irritant receptors was reduced, or both. The depression of activity at larger lung

volumes is unlikely to have been due to changes in functional residual capacity, since lung compliance did not change in this group of animals. The decrease in ratio of stretch to irritant receptor discharge could be due to an increase in numbers or sensitivity of lung irritant receptors in the exposed rabbits ; however the concentration of SO_2 used is a weak stimulant of irritant receptors [17] and is known to inhibit acutely pulmonary stretch receptors [8, 28]. The test stimuli used for irritant receptors were strongly supra-maximal. Observer bias is a possible influence on receptor ratio, but the possibility of using this method of analysis was apparent only after all the records of vagal afferent single-fibre activity had been made. The presence of apparently damaged stretch receptors supports the view that SO_2 has a harmful action on them.

(2) The Breuer-Hering inflation reflex was reduced at larger lung volumes, direct evidence that stretch receptors were inhibited.

(3) Exposure to SO_2 increased total lung resistance. The inflation reflex is bronchodilator and its abolition can increase resistance [12, 18]. Stimulation or sensitization of irritant receptors may also increase total lung resistance [17, 30].

(4) There was an increased breathing frequency response to CO_2. Abolition of the inflation reflex is known to have this effect [19, 22]. Acute exposure of rabbits to SO_2 increases the breathing frequency response to CO_2 by paralysis of pulmonary stretch receptor activity [8].

Although in general our results are consistent with the view that SO_2 - induced bronchitis inhibits pulmonary stretch receptor activity, there are clear problems that require discussion. The difference between results for the single low-dose and multiple high-dose experiments may be because the former was a more powerful irritant stimulus. Thus in the multiple low-dose exposure group total lung resistance was not changed, individual pulmonary stretch receptors were not inhibited, and the decrease in stretch-to-irritant receptor ratio was smaller, compared with the low-dose group. After exposure in the low and high-dose groups, breathing frequency slowed ; this result is consistent with inhibition of stretch receptors [8].

Although there was a potentiation after exposure to SO_2 of the breathing frequency response to CO_2, pneumothorax and ammonia, stimuli all known to act via lung irritant receptors [2, 8, 23], this was not seen with histamine and phenyl diguanide. Phenyl diguanide stimulates J-receptors in the alveolar wall [20], and histamine stimulates both irritant receptors [2, 16] and J-receptors in the alveolar wall [20] and bronchial wall [5]. It is possible that exposure to SO_2 in our experiments had no effect on the breathing frequency response to stimulation of J-receptors, and that the two drugs were acting by this mechanism.

Interpretation of our results in terms of lung pathology is limited to the histological observation that, with light microscopy, airway tissues appeared normal apart from loss of cilia from the epithelium. The depth of penetration of SO_2 into the lungs is a key question in studies of this type [26] as is the possibility of hypersecretion of mucus and the protective effect that this mucus may have against the action of SO_2. Further studies would be needed to clarify these problems.

In spite of several equivocal results, the most consistent explanation of our results is that chronic exposure of rabbits to SO_2 specifically inhibits pulmonary stretch receptor activity with no measured effect on lung irritant and J-receptor activity. This specificity of action is even more pronounced in experiments with acute exposure of rabbits to SO_2 [8]. Since there are wide differences in the strength of the Breuer-Hering reflex in different species [29], it is not justifiable to apply these results quantitatively to species other than the rabbit. In particular, any comparison between our results and studies on human bronchitis must be tenuous, since aetiologies may be greatly different. However, some human chronic bronchitics do seem to have abnormal patterns of breathing and responses to CO_2, especially in relation to frequency and pattern of breathing [11, 14], and the possibility that the disease is causing derangement of function of lung nervous receptors must be considered. If pulmonary stretch receptors are inhibited in human chronic bronchitis, this could enhance nervously-mediated bronchoconstriction, since these receptors cause a reflex bronchodilation ; the fact that airways resistance in chronic bronchitis is considerably reduced by atropine [3] is consistent with the presence of this mechanism. The rapid recovery (seven days) of function after exposure to SO_2 in our experiments clearly does not apply to human chronic bronchitis.

CONCLUSIONS

Rabbits were chronically exposed to sulphur dioxide, to an extent that sometimes increased total lung resistance to airflow, but produced no histological damage in the tracheobronchial epithelium apart from a disappearance of cilia. This treatment reduced the strength of the Breuer-Hering reflex, and produced changes in the pattern of breathing consistent with this effect. Recordings of single-fibre activity from pulmonary stretch receptors showed that their sensitivity was reduced. Breathing responses to pneumothorax and to inhaled carbon dioxide or ammonia also suggested that pulmonary stretch receptors and Breuer-Hering reflex activity was depressed. Other lung reflexes, such as those induced by intravenous injections of histamine or phenyl diguanide possibly acting on lung J-receptors, were little affected. The results raise the question whether the changes in the nervous control of breathing in SO_2-induced chronic bronchitis in rabbits may apply also to other forms of chronic bronchitis, including that in man.

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RÉSUMÉ

Des lapins ont été exposés à l'anhydride sulfureux à des concentrations de 300 ppm pendant trois heures ou de 150 ppm pendant douze périodes de trois heures. Après l'exposition, ont été mesurés : 1. la mécanique ventilatoire, 2. le réflexe d'inflation de Hering-Breuer, 3. les réponses ventilatoires à l'inhalation de CO_2 et d'ammoniaque, à un pneumothorax, ainsi qu'à des injections intraveineuses d'histamine et de phényl diguanide, 4. l'activité des récepteurs pulmonaires à la tension et aux irritants par enregistrement d'une fibre isolée.

Les résultats montrent que l'exposition chronique au SO_2 inhibe l'activité des récepteurs de tension, ainsi que ses effets réflexes ; on peut se demander si ce mécanisme joue un rôle dans le contrôle de la respiration chez les malades bronchiteux chroniques.

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Unusual response of anaesthetised pigs to asphyxia

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The effects of vagosympathectomy, asphyxia, hypoxia and hypercapnia on the breathing of anaesthetised pigs are described. Vagosympathectomy caused changes in cardiovascular variables and in the pattern of breathing characteristic of the loss of stretch receptor activity. After vagosympathectomy the linear relationship between t_I and t_E was abolished.

Hypoxia in intact animals produced changes in minute ventilation by peripheral chemoreceptor drive. When this drive was removed by vagosympathectomy the central depressing effects of hypoxia were revealed as a slowing of breathing and reduction in minute volume. The central depressing effect of hypoxia on respiration was very potent in the pig and very clearly seen in asphyxia. Vagosympathectomy caused a reduction in frequency of breathing and respiratory arrest occurred when a dead space of only moderate size was used. Breathing slowed from the moment of connection of the dead space to produce respiratory arrest within 2 min. The pig lung has been considered similar to the human lung on morphometric and physiological grounds but these results show that there are very important species differences in response to asphyxia.

IN AIR BREATHING animals section of the vagus nerves invariably modifies the pattern of breathing. There is usually a reduction in rate and an increase in depth of breathing (Karczewski and Widdicombe 1969). These changes are due to the loss of afferent activity from lung receptors. Of all the common experimental animals only the guinea pig shows immediate pathological changes to vagosympathectomy, fatal lung oedema developing within a period of hours. Vagosympathectomy does not usually interfere with an animal's ability to maintain normal blood gas tension (Richardson and Widdicombe 1965) nor to respond to the inhalation of hypercapnic, hypoxic or asphyxic mixtures. In this paper we describe how vagosympathectomy in the pig specifically destroys the normal response to asphyxia while leaving the responses to hypercapnia and hypoxia intact.

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Materials and methods

Seven Large White pigs of either sex, weighing 10 to 25 kg, were anaesthetised with pentobarbitone sodium (Nembutal, Abbott); 30–40 mg/kg was injected intravenously into the cranial caval vein through the jugular sulcus. The animals were premedicated with 0·05 ml/kg of 1 per cent propionylpromazine given intramuscularly, which quietened them sufficiently to enable the anaesthetic injection to be made with minimal restraint by hand.

Supplementary doses of pentobarbitone sodium, when necessary, were given through a catheter inserted in the femoral vein. Depth of anaesthesia was monitored using rate and depth of respiration and heart rate. These, of course, changed after vagosympathectomy and care was taken not to cut the nerves immediately after a dose of anaesthetic. The pigs were tied out supine. Systemic arterial blood pressure was recorded from the right femoral artery through a polyethylene catheter by a fluid filled capacitance manometer (Bell and Howell 4–422). Mean systemic arterial blood pressure was determined as diastolic pressure plus one third of the pulse pressure.

A tracheal cannula was inserted just below the cricoid cartilage. Transpulmonary pressure (tracheal minus intrapleural pressure) was measured between an air-filled polyethylene catheter tied into the lower right intercostal space and a wide bore needle inserted into the tracheal cannula, using a differential capacitance manometer (Bell and Howell 4104). Airflow was measured using a Fleisch No 2 pneumotachograph head of internal diameter 28 mm attached to the tracheal cannula and a differential conductance transducer (Statham 10987). Tidal volume was obtained by electrical integration. The animal's temperature was measured with a rectal probe and maintained by an electric blanket. Records of systemic arterial blood pressure, flow, tidal volume and transpulmonary pressure were recorded on ultraviolet-sensitive paper by a Honeywell UV31 oscillograph for later measurement. The variables were also displayed on an oscilloscope (Tektronix 5103).

Vagosympathectomy was performed bilaterally in the midcervical region. In this region the vagus nerve joins the sympathetic trunk and they are held in close association by a common connective tissue sheath down to the level of the seventh cervical vertebra. In experiments involving vagosympathectomy 20 to 30 min were allowed to elapse after cutting the nerves before further procedures were carried out. Hypercapnia and hypoxaemia were produced with gas mixtures obtained from commercial cylinders and fed into a T piece inserted rostral to the Fleisch-Head. The CO_2 concentrations were 4 and 8 per cent in air; the O_2 was 10 per cent in N_2. Each gas was given for 10 min which produced a stable response. Between 10 and 15 min were allowed between administration of gas mixtures. Arterial P_{O_2} and P_{CO_2} were measured at the end of the period of gas administration. Pa_{O_2} and Pa_{CO_2} of 1·55 ml blood samples taken from the blood pressure cannula in the right femoral artery were measured by Blood Gas Analyser 413 (Instrumentation Laboratory). End-tidal CO_2 was measured with an infra-red absorption meter (Beckman Medical Gas Analyser LB2), connected distally to the pneumotachograph head and sampling at 500 ml/min. Asphyxia was induced by making the animals rebreathe through wide-bore tubes of 270 and 390 ml. Each pig was subjected to the whole experimental procedure. No systematic change in sensitivity, which could be attributed to the progressive effects of sustained anaesthesia, was observed. As each animal acted as its own control for each acute experimental procedure, separate studies to determine the effects of duration of anaesthesia and the total dose of barbiturate used were not considered necessary.

Results

The tables summarise the results obtained in spontaneously breathing pigs before and after vagosympathectomy. The results are given as mean ± SE of absolute values of control, and absolute and percentage values for changes after vagosympathectomy. Student's *t* test for significance was applied.

Pattern of breathing

Bilateral cervical vagosympathectomy caused characteristic slower deeper breathing, without any significant change in minute ventilation.

End tidal CO_2, $PaCO_2$ and PaO_2 were not significantly changed after vagosympathectomy (Table 1). When inspiratory and expiratory duration (t_I and t_E) were compared for individual breaths at different levels of hypercapnia a fixed relationship was seen when the vagosympathetic trunks were intact. When the vagosympathetic trunks were cut this was abolished, t_I remaining fixed for different values of t_E (Fig 1). This fixation of t_I after vagosympathectomy is also seen when t_I is plotted against tidal volume (V_T) for individual breaths (Fig 2).

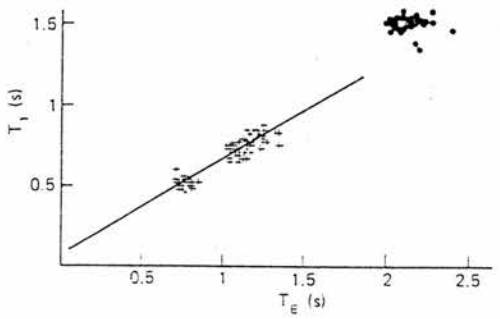

FIG 1: Relationship between t_I and t_E in spontaneous breathing (+) and vagosympathectomised pig (●)

TABLE 1: Effect of bilateral cervical vagosympathectomy on some respiratory and circulatory variables. Values are means (± SE) and are expressed as absolute values for controls and as absolute and % values for changes after vagosympathectomy. Statistical values apply to the significance of the mean changes

Variable	No	Control	Change	
			Absolute	%
Resp frequency (per min)	7	30·12 ± 4·12	−12·59 ± 4·73*	−41·80 ± 11·06*
Tidal volume (ml)	7	170·23 ± 22·88	+37·32 ± 30·14	+21·92 ± 20·03
Volume minute (litre/min)	7	4·722 ± 0·53	−1·25 ± 0·64	−26·41 ± 11·30
End-tidal CO_2 ± (mmHg)	7	39·38 ± 1·48	+2·97 ± 2·71	+7·54 ± 7·05
Pa CO_2 (mmHg)	5	40·50 ± 3·36	+0·60 ± 4·96	+1·48 ± 12·32
Pa O_2 (mmHg)	5	71·10 ± 7·79	−1·35 ± 10·02	−1·90 ± 13·93
Arterial BP (mmHg)	7	77·14 ± 4·42	−11·17 ± 6·48	−14·48 ± 7·86
Heart rate (per min)	6	140·66 ± 12·44	−23·52 ± 14·88	−16·72 ± 9·37

* $P < 0·05$

TABLE 2: Effect of 4% CO_2 on some respiratory and circulatory variables before and after vagosympathectomy. Values are means (± SE) and are expressed as absolute values for controls and as absolute and % values for changes after vagosympathectomy. Statistical values apply to the significance of the mean change

Variable	No		Control	Change Absolute	Change %
Resp frequency (per min)	7	N	30·95 ± 3·14	+5·12 ± 5·69	+16·54 ± 19·38
	7	VC	17·53 ± 2·31	−2·01 ± 2·78	−11·48 ± 14·60
Tidal volume (ml)	7	N	173·90 ± 23·67	+27·68 ± 32·45	+15·92 ± 20·29
	7	VC	207·55 ± 19·62	+38·17 ± 33·88	+18·39 ± 17·39
Volume minute (litre/min)	7	N	5·496 ± 1·14	+1·05 ± 1·46	+27·20 ± 31·21
	7	VC	3·474 ± 0·36	+0·24 ± 0·55	+6·82 ± 16·28
End-tidal CO_2 (mmHg)	7	N	38·23 ± 1·93	+4·48 ± 2·80	+11·71 ± 7·74
	7	VC	42·35 ± 2·27	+10·30 ± 3·13**	+24·30 ± 8·93**
Pa CO_2 (mmHg)	5	N	40·63 ± 2·34	—	—
	5	VC	41·10 ± 3·64	—	—
Pa O_2 (mmHg)	5	N	77·10 ± 6·69	—	—
	5	VC	69·75 ± 6·29	—	—
Arterial BP (mmHg)	7	N	77·55 ± 4·20	+2·42 ± 5·72	+3·12 ± 7·50
	7	VC	65·97 ± 4·74	−0·95 ± 6·63	−1·44 ± 9·97
Heart rate (per min)	7	N	147·42 ± 11·62	+6·15 ± 16·48	+4·17 ± 11·41
	7	VC	117·14 ± 8·15	−5·48 ± 12·69	−4·67 ± 10·62

** $P < 0·01$ N = intact VC = vagosympathetics cut

TABLE 3: Effect of 8% CO_2 on some respiratory and circulatory variables before and after vagosympathectomy. Values are means (± SE) and are expressed as absolute values for controls and as absolute and % values for changes after vagosympathectomy. Statistical values apply to the significance of the mean change

Variable	No		Control	Change Absolute	Change %
Resp frequency (per min)	7	N	30·95 ± 3·14	+14·46 ± 5·12*	+46·68 ± 19·82*
	7	VC	17·53 ± 2·31	+0·04 ± 2·87	+0·22 ± 16·40
Tidal volume (ml)	7	N	173·90 ± 23·67	+79·90 ± 47·84	+45·94 ± 31·08
	7	VC	207·55 ± 19·62	+140·30 ± 56·31*	+67·70 ± 29·96*
Volume minute (litre/min)	7	N	5·496 ± 1·15	+5·484 ± 2·00*	+99·78 ± 52·52*
	7	VC	3·474 ± 0·36	+2·37 ± 0·74**	+68·25 ± 25·62**
End-tidal CO_2 (mmHg)	7	N	38·23 ± 1·93	+20·09 ± 3·50**	+52·55 ± 10·87**
	7	VC	42·35 ± 2·27	+28·17 ± 3·07**	+66·52 ± 10·19**
Pa CO_2 (mmHg)	5	N	40·63 ± 2·34	+8·47 ± 2·39**	+20·85 ± 7·08**
	5	VC	41·10 ± 3·64	+19·56 ± 4·77**	+47·59 ± 15·08**
Pa O_2 (mmHg)	5	N	77·10 ± 6·69	+20·60 ± 12·98	+26·72 ± 18·14
	5	VC	69·75 ± 6·29	+10·41 ± 17·90	+14·92 ± 26·17
Arterial BP (mmHg)	7	N	77·55 ± 4·20	+3·47 ± 7·08	+4·47 ± 9·27
	7	VC	65·97 ± 4·74	+1·15 ± 6·98	+1·74 ± 10·67
Heart rate (per min)	7	N	147·42 ± 11·62	−1·59 ± 15·94	−1·08 ± 10·75
	7	VC	117·14 ± 8·15	+14·52 ± 16·36	+12·40 ± 14·41

** $P < 0·01$, * $P < 0·05$ N = intact VC = vagosympathetics cut

Hypercapnia

Tables 2 and 3 summarise the results of administering 4 and 8 per cent CO_2 in air.

With intact vagosympathetic trunks 4 or 8 per cent CO_2 in air increased respiratory frequency, tidal volume and minute ventilation. After vagosympathectomy changes in minute ventilation due to hypercapnia were brought about by changes in tidal volume alone (Fig 2).

Hypoxia

Hypoxia was produced with 10 per cent O_2 in N_2. The results are summarised in Table 4.

Asphyxia

Dead spaces of 270 and 390 ml were used. The results are summarised in Tables 5 and 6. In the intact animals both large and small dead spaces

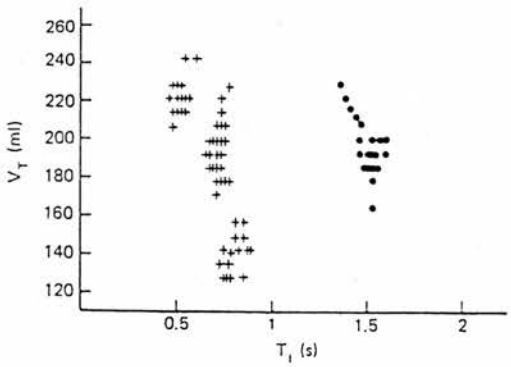

FIG 2: Relationship between VT and TI in spontaneous breathing (+) and vagosympathectomised pig (●)

increased respiratory frequency and tidal volume. In vagosympathectomised animals, on the other hand, the small dead space produced a slowing of respiration and small increase in tidal volume. These combined to produce a small decrease in minute ventilation. The slowing effect of asphyxia was so great in the vagosympathectomised animals that with a 390 ml added dead space breathing slowed from the moment of connection of the dead space to produce respiratory arrest within 2 min.

Discussion

As with other species, vagosympathectomy in pigs caused changes in blood pressure and heart rate (Tables 1–6) and changes in the pattern of breathing (Clark and von Euler 1970) characteristic of the loss of stretch receptor activity. Breathing became slower and deeper. The linear relationship between t_I and t_E was abolished (Fig 1). This was very clearly seen in the response to hypercapnia where increased minute ventilation was brought about exclusively by changes in tidal volume after vagosympathectomy. Despite these changes in the pattern of breathing there was little change in end tidal CO_2 under normocapnic conditions after vagosympathectomy, indicating that alveolar ventilation could still be maintained. These findings are similar to those of Weimer and Kiwull (1965) and Richardson and Widdicombe (1965) for anaesthetised and unanaesthetised rabbits subjected to vagotomy only. In hypercapnia however the restriction placed on frequency of breathing by vagosympathectomy reduced the animal's ability to maintain normal blood gas tensions. Hypoxia in intact animals produced large changes in minute ventilation by peripheral chemoreceptor drive. This hypoxic drive arises from the carotid and aortic bodies (described in the pig by Watzka 1931). Afferent fibres from the carotid bodies join the glossopharyngeal nerve via the sinus nerve. Afferent fibres from the aortic bodies enter the vagosympathetic trunk, and it is this pathway that was interrupted by vagosympathectomy. When this drive was removed by vagosympathectomy the central depressing effects of the hypoxia were revealed as a slowing of breathing and reduction in minute volume. The central depressing effect of hypoxia on respiration was very potent in the pig and very clearly seen

TABLE 4: Effect of 10% O_2 on some respiratory and circulatory variables before and after vagosympathectomy. Values are means (± SE) and are expressed as absolute values for controls and as absolute and % values for changes after vagosympathectomy. Statistical values apply to the significance of the mean change

Variable	No		Control	Change Absolute	Change %
Resp frequency (per min)	7	N	30·74 ± 4·68	+10·23 ± 6·08	+33·29 ± 23·92
	7	VC	16·57 ± 1·97	−1·07 ± 4·18	−6·46 ± 24·85
Tidal volume (ml)	7	N	172·91 ± 22·83	−26·56 ± 27·05	−15·38 ± 13·97
	7	VC	211·93 ± 16·89	+15·21 ± 29·73	+7·18 ± 14·36
Volume minute (litre/min)	7	N	5·08 ± 0·96	+0·91 ± 1·31	+17·96 ± 28·43
	7	VC	3·44 ± 0·44	+0·21 ± 1·21	+6·16 ± 35·34
End-tidal CO_2 (mmHg)	7	N	40·15 ± 2·31	−10·20 ± 3·21**	−25·40 ± 7·02**
	7	VC	44·45 ± 2·09	−8·69 ± 3·19*	−19·55 ± 6·60*
Pa CO_2 (mmHg)	3	N	43·08 ± 2·77	−8·63 ± 3·57	−20·02 ± 7·34
	4	VC	42·85 ± 2·84	−0·52 ± 4·12	−1·21 ± 9·57
Pa O_2 (mmHg)	3	N	73·30 ± 9·29	−37·23 ± 10·63*	−50·79 ± 9·41*
	4	VC	62·12 ± 8·68	−33·00 ± 11·21*	−53·12 ± 13·17*
Arterial BP (mmHg)	6	N	82·33 ± 4·36	−5·62 ± 7·14	−6·83 ± 8·45
	7	VC	62·15 ± 3·73	−11·42 ± 5·13	−18·37 ± 7·50
Heart rate (per min)	6	N	142·50 ± 10·78	+12·50 ± 18·20	+8·77 ± 13·17
	6	VC	116·25 ± 8·84	+23·75 ± 12·87	+20·43 ± 12·19

** $P < 0·01$ * $P < 0·05$ N = intact VC = vagosympathetics cut

TABLE 5: Effect of dead space (270 ml) on some respiratory and circulatory variables before and after vagosympathectomy. Values are means (± SE) and are expressed as values for controls and as absolute and % values for changes after vagosympathectomy. Statistical values apply to the significance of the mean change

Variable	No		Control	Change Absolute	Change %
Resp frequency (per min)	7	N	31·00 ± 5·33	+6·46 ± 6·85	+20–86 ± 25·00
	5	VC	16·69 ± 3·11	−1·83 ± 4·27	−10·96 ± 24·12
Tidal volume (ml)	7	N	168·47 ± 24·01	+75·89 ± 29·21*	+45·01 ± 22·90*
	5	VC	230·74 ± 22·27	+6·46 ± 31·90	+2·80 ± 14·02
Volume minute (litre/min)	7	N	4·760 ± 0·70	+4·41 ± 1·47**	+92·65 ± 39·15**
	5	VC	3·734 ± 0·70	−0·18 ± 1·13	−4·87 ± 29·57
End-tidal CO_2 (mmHg)	7	N	38·09 ± 1·27	+20·81 ± 4·34**	+54·65 ± 12·05**
	5	VC	43·74 ± 1·51	+12·76 ± 3·62*	+20·10 ± 8·74*
Pa CO_2 (mmHg)	5	N	37·92 ± 2·74	—	—
	2	VC	41·97 ± 4·08	—	—
Pa O_2 (mmHg)	5	N	71·48 ± 6·42	—	—
	2	VC	51·00 ± 4·00	—	—
Arteri.l BP (mmHg)	6	N	80·65 ± 6·30	−4·72 ± 8·72	−5·84 ± 10·49
	5	VC	64·95 ± 2·81	−11·68 ± 11·13	−17·98 ± 16·96
Heart rate (per min)	5	N	149·00 ± 14·76	+15·17 ± 19·28	+10·18 ± 13·73
	4	VC	128·75 ± 6·25	−0·42 ± 11·91	−0·33 ± 9·24

** $P < 0·01$ * $P < 0·05$ N = intact VC = vagosympathetics cut

TABLE 6: Effect of dead space (390 ml) on some respiratory and circulatory variables before and after vagosympathectomy. Values are means (± SE) and are expressed as absolute values for controls and as absolute and % values for changes after vagosympathectomy. Statistical values apply to the significance of the mean change

Variable	No		Control	Change Absolute	Change %
Resp frequency (per min)	7	N	31·00 ± 5·33	+7·12 ± 9–81	+22·97 ± 33·96
	5	VC	16·69 ± 3·11	—	—
Tidal volume (ml)	7	N	168·47 ± 24·01	+09·68 ± 29·55*	+53·83 ± 24·19*
	5	VC	230·74 ± 22·27	—	—
Volume minute (litre/min)	7	N	4·760 ± 0·70	+4·99 ± 2·62*	+105·02 ± 54·21*
	5	VC	3·734 ± 0·70	—	—
End-tidal CO_2 (mmHg)	7	N	38·09 ± 1·27	+28·73 ± 5·02**	+75·44 ± 14·04**
	5	VC	43·74 ± 1·51	—	—
Pa CO_2 (mmHg)	5	N	37·92 ± 2·74	+19·22 ± 6·61**	+05·68 ± 19·25**
	2	VC	41·97 ± 4·08	—	—
Pa O_2 (mmHg)	5	N	71·48 ± 6·42	−20·54 ± 11·11	−28·74 ± 14·21
	2	VC	51·00 ± 4·00	—	—
Arterial BP (mmHg)	6	N	80·65 ± 6·30	−11·33 ± 10·00	−14·05 ± 11·75
	5	VC	64·95 ± 2–81	—	—
Heart rate (per min)	5	N	149·00 ± 14·76	+4·00 ± 28·23	+2·68 ± 19·08
	4	VC	128·75 ± 6·25	—	—

** $P < 0·01$ * $P < 0·05$ N = intact VC = vagosympathetics cut

in asphyxia. We produced asphyxia by causing our animals to rebreathe from a tube of the same proportions as used by Dixon *et al* (1974) to produce asphyxia in cats. After vagotomy Dixon *et al* (1974) observed increases in breathing frequency while tidal volume and end tidal CO_2 were reduced. Vagosympathectomy in our rebreathing experiments caused a reduction in frequency, probably due to hypoxia of the respiratory centres, which was probably responsible for the respiratory arrest seen after vagosympathectomy and asphyxia produced by a dead space of only moderate size (390 ml). This response is even more remarkable when one considers that the effect of tracheal cannulation or intubation was to reduce the natural dead space.

Asphyxia can be considered to be made up of hypercapnic and hypoxic drives. The depressing effects of asphyxia on respiration can be more clearly

understood if these two drives are analysed separately. The intact animals' response to hypercapnia consisted of an increase in minute volume comprising in almost equal parts, rises in frequency and tidal volume. Vagosympathectomy greatly reduced the animals' ability to increase respiratory rate and so effectively halved the response to the hypercapnic component of asphyxia. Similarly the normal response to hypoxia, which was largely an increase in frequency, was abolished by vagosympathectomy. Suppression of a large part of the hypercapnic and hypoxic responses to asphyxia revealed the central depressing effects of asphyxia which were so great in the case of the larger dead space that they arrested breathing after vagosympathectomy.

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The role of conducting airways in partial separation of inhaled gas mixtures.

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Role of conducting airways in partial separation of inhaled gas mixtures

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HORSFIELD, KEITH, ANDREW DAVIES, AND GORDON CUMMING. *Role of conducting airways in partial separation of inhaled gas mixtures.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 43(3): 391–396, 1977. – A positive (hollow) cast of the bronchial tree was made from a pig's lung. Gas mixtures containing sulfur hexafluoride (SF_6) and helium (He), and SF_6 and argon (Ar), were blown down the cast at two different flows, the cast having first been filled with air. Gas was sampled by a mass spectrometer probe from 1-mm-diam branches situated on short, medium, and long pathways. The front of the SF_6 appeared in advance of the fronts of the He and the Ar. This relative advancement was greater *a*) with the SF_6/He mixture than with the SF_6/Ar mixture: *b*) at slower flows; and *c*) on longer pathways. With reverse flow up the cast using SF_6/He there was little difference between the arrival times of the two gas fronts at either flow. These results could be explained by the effects of Taylor dispersion on gases having different diffusion coefficients.

pig bronchial tree; Taylor dispersion; gas diffusion

THE EXTENT TO WHICH LIMITATION of molecular diffusion impairs the mixing of gases in the pulmonary airspaces has been the subject of considerable debate. Georg et al. (5) investigated this process by including in an inspirate three gases of differing diffusion coefficients, namely helium (He), neon, and sulfur hexafluoride (SF_6). On expiration relatively more SF_6 appeared early in the expirate and relatively more He appeared late. This was interpreted as being due to the more rapidly diffusible He penetrating further into the lung, and thereby lowering its concentration in the proximal airways. Experiments by Cumming et al. (3), Power (8), and Hogg et al. (6) have yielded similar results. Hogg et al. (6) also produced partial airway blockage of excised lungs by insufflating with beads. After this they found that both end-expiratory and residual gas contained relatively more SF_6. This they attributed to the dispersion mechanism described by Taylor (10) who analyzed both mathematically and experimentally the dispersion of a solute in a solvent flowing through a tube. The dispersion results from the combined effects of a parabolic velocity profile and of radial diffusion.

Consider a long tube, initially containing pure solvent, into which flows solvent containing a diffusible solute. A parabolic velocity profile will develop (Fig. 1*A*) and this will tend to form a parabolic concentration profile at the front of the solute. In the region of this concentration profile the solvent at the center of the tube contains more solute than does that at the periphery, so the solute diffuses radially towards the periphery (Fig. 1*B*) thereby changing the shape of the concentration profile (Fig. 1, *C* and *D*). This solute moves from the more rapidly flowing central parts of the stream to the slower flowing peripheral parts, so that the longitudinal dispersion of the solute is less than it would have been had not radial diffusion occurred (Fig. 1*D*). Taylor showed that when axial diffusion is slow enough to be disregarded the resulting dispersion is equivalent to an apparent diffusion with an apparent diffusion coefficient k, where $k = r^2v^2/192D$, r = radius of tube, v = maximum velocity of flow in the center of the tube, and D = molecular diffusion coefficient of the solute. The coefficient k is inversely proportional to D so that the more diffusive the solute the less is the longitudinal dispersion and the shorter the concentration profile. This dispersion occurs relative to a plane at right angles to the axis of the tube which moves along the tube with velocity $v/2$. The 50% point of the concentration profile is normally situated in this plane (Fig. 2). However, if axial diffusion is sufficiently great that it cannot be disregarded the apparent diffusion coefficient requires a correction, in which case $k = D + r^2v^2/192D$ (1). As the ratio of axial molecular diffusion to velocity increases, the concentration profile shown in Fig. 1*D* becomes flatter and approaches in shape that which would result from molecular diffusion alone. The more diffusible substance may then undergo the greater longitudinal dispersion.

The purpose of the present study is to determine the part played by the conducting airways in the partial separation of gas mixtures during inspiration and expiration, and whether Taylor dispersion or the physical properties of the gases used might explain any such separation.

METHODS

A positive (hollow) cast of the airways of a pig's lung was made as described by Davies (4). Briefly, a wax cast of the airways of the inflated lung is made first, and the tissues are then corroded in hydrochloric acid. The negative wax cast is coated with colloidal silver and then electroplated with silver. Finally the wax is removed from within the hollow cast by melting it and blowing it out.

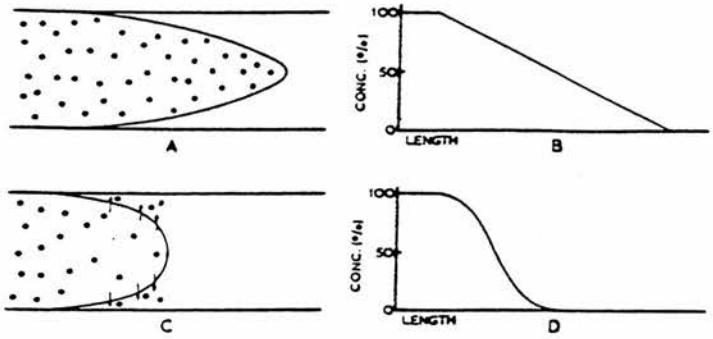

FIG. 1. *A*: Laminar flow in a cylindrical tube without Taylor dispersion. Dots represent solute in solvent flowing from left to right. Parabolic concentration profile develops at front of solute. *B*: Concentration/length plot resulting from *A*. Concentration represents mean value across a section of tube at right angles to its axis. *C*: Laminar flow in cylindrical tube with Taylor dispersion. Solute diffuses radially (arrows) at same time as concentration profile develops. *D*: Concentration/length plot resulting from *C*. Note that concentration profile extends over shorter length of tube than in *B* for a given duration of flow.

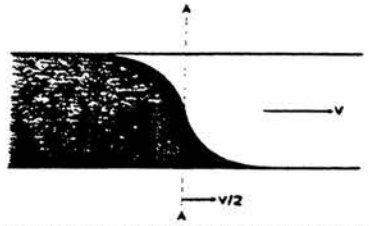

FIG. 2. Forward movement of concentration profile developed by Taylor dispersion. Maximum velocity of flow, v, is in center of tube but plane A–A moves at only half this velocity, $v/2$. Concentration profile of solute develops in relation to plane A–A as if it were stationary with solute diffusing axially according to usual laws of diffusion, but with modified diffusion coefficient (see text).

The cast of the main bronchus was fixed to a plastic cannula to facilitate handling, clamping, and the connection of tubing without damage.

Experiments

When molecular diffusion operates on the front of a gas mixture flowing through a tube, the longitudinal dispersion is related to diffusivity in a biphasic manner: if velocity is low compared to diffusivity the most diffusible gas becomes most dispersed, but when the velocity is greater the most diffusible gas may be least dispersed. If viscosity could in some way affect the components of the gas mixture independently then separation might result from this; however, gas mixtures behave as one with respect to viscous effects, so this is an unlikely possibility. The possible effects of density are difficult to anticipate, and furthermore are difficult to differentiate from the effects of diffusion, as both are related to molecular weight.

Three sets of experiments were performed. In the first a mixture of air, SF_6, and He was blown down the cast ("inspiration") and the gas sampled just within the open end of several peripheral airways. This showed that He was delayed relative to the SF_6, a result which could be due either to Taylor dispersion (He is more diffusive) or to some effect which is dependent upon viscosity (He is more viscous).

The second set of experiments was designed to differentiate between these two possible explanations. The experiments were similar to the first set except that a gas mixture containing SF_6 and Ar was substituted for that containing SF_6 and He. Ar is less diffusive than He but more viscous, so that if Ar were more delayed than He viscous effects would be a possible explanation, while if it were less delayed then Taylor dispersion would be the more likely. We were unable to devise an experimental protocol which would differentiate between the effects of Taylor dispersion and differences in density.

In the third set of experiments the direction of flow was reversed ("expiration"), using the SF_6 and He mixture.

Experiment 1. The setup is shown in Fig. 3. A mixture of air, SF_6, and He, approximately 60:20:20 by volume, was made up in a Douglas bag and thoroughly mixed. A three-way tap, a pneumotachograph, and the cast were connected in series with the bag. The side arm of the three-way tap was connected to an air pump so that the cast could be cleared of residual gas mixture after each run. Gas entering the cast was sampled just before the main bronchus by a mass spectrometer probe. A diaphragm with a slit-like opening mimicking the larynx was inserted in the lumen of the tubing between the pneumotachograph and the cast in order to produce turbulent flow. That this was effective was checked by observing its effect on streams of smoke passing through a transparent tube. Outflowing gas from the cast was sampled just inside the open ends of bronchi about 1-mm internal diameter. Gas concentrations were measured with a Centronics quadrupole mass spectrometer with a gas sample flow rate of 3 ml/min and the concentrations and flow rates were recorded with a Medelec recorder. Flow was achieved by placing a weight on the Douglas bag and then opening the tap to the cast.

Triplicate runs were made at each of two flows, 16 l/min ("fast flow") and 8 l/min ("slow flow"). Gas was sampled at the inlet to the cast and the transient concentrations of SF_6 and He measured as the front went through. Concentrations and flow rate were recorded on UV paper running at 10 cm/s.

Next, six peripheral branches were selected such that two were situated on short pathways from the main bronchus, two on medium length pathways, and two on long pathways. Duplicate runs were made at each flow.

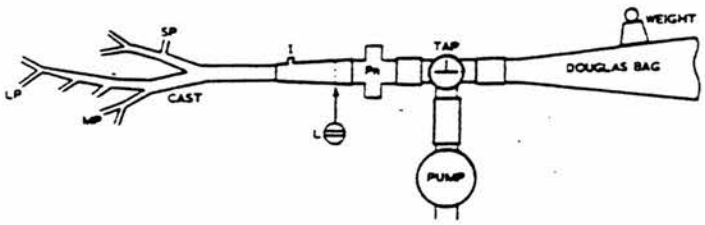

FIG. 3. Diagram of apparatus used in *expts 1* and *2*. I = gas sample point for input; L = diaphragm with transverse opening to imitate larynx; Pn = pneumotachograph; SP = short pathway; MP = medium pathway; LP = long pathway (see text).

sampling at the branch as the front of the gas mixture came through.

The recordings were measured in the following way. A sample of gas mixture was measured directly from the Douglas bag and the signals obtained were taken to be 100% concentration. The times of appearance on the experimental record of 10%, 25%, 50%, 75%, and 85% signals were measured for each gas, arbitrarily defining the 10% He point as zero time. From the flow record, which reached a constant value before the gas mixture entered the cast, the volume which had passed in the measured time was calculated. The final results were expressed as percent concentration against volume.

Experiment 2. This was similar to *experiment 1* but with the following exceptions: Ar was substituted for He, four runs were made at each flow, and only one short, one medium, and one long pathway were studied. Rather slower flows were chosen, 10 l/min for fast flow and 4 l/min for slow flow, because the effects observed in *experiment 1* were found to be more marked at slower flows.

Experiment 3. Flow in the expiratory direction was obtained by enclosing the cast in a box with the cannula passing through the lid, filling the box with the gas mixture, and then raising the pressure (Fig. 4). The box was made of acrylic plastic (Lucite); it had a tight-fitting lid and various access holes. The cannula attached to the cast passed through a rubber bung, which in turn sealed the hole in the lid, and a pneumotachograph was attached to the open end of the cannula. A Douglas bag and a three-way tap were attached to a short length of pressure tubing passing through the side of the box. A similar tube on the other side of the box permitted gas samples to be obtained. By temporarily fixing another bag to this tube, mixing of the gas mixture throughout the system was achieved by squeezing each bag in turn. This process was aided by an electric fan placed on the bottom of the box. At the end of this maneuver the box and the bag contained the gas mixture and the extra bag was removed.

Input samples were obtained by weighting the Douglas bag, opening the tap, and sampling from the other outlet tube. Duplicate readings were taken at flows of 9 l/min. Before each expiratory flow run a small quantity of air was blown down the cast in order to clear out any residual gas mixture. The small amount of air entering the box through the cast was mixed by the action of the fan. Immediately the clearing flow was stopped the tap

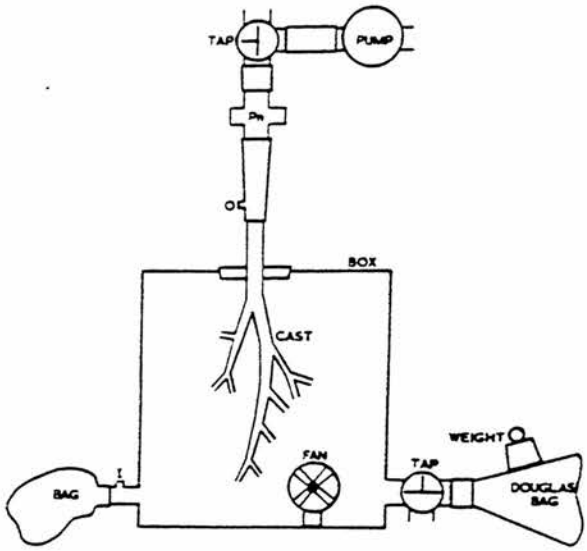

FIG. 4. Diagram of apparatus used in *expt 3*. I = gas sample point for input; Pn = pneumotachograph; O = gas sample point for output (see text).

on the weighted Douglas bag was opened and gas flowed out of the box through the cast. It was sampled from the tubing attaching the pneumotachograph to the cannula. Duplicate runs were made at each flow. In this experiment different path lengths cannot of course be utilized because the gas which appears at the outlet represents a mixture of that coming from all the pathways.

RESULTS

The results of *experiments 1, 2,* and *3* are shown in Figs. 5, 6, and 7, respectively, and in Table 1. Each point on the graphs shows the mean value obtained from the replicate runs under a given set of conditions. The input concentration plots are shown on the left of each row of plots. As is to be expected, the rise in concentration with volume is not a square wave. Furthermore SF_6, the densest gas in the mixture, appears last. These features result from the combined effects of flow through the tubing between bag and sampling point, and the mass spectrometer and its sample line system. Since the gas mixture must traverse these in every run, any differences between the input and output curves must be due to the effect of the cast. It must be pointed out that because each set of experiments was performed on a different day using different sample lines it is not possible to make direct comparisons between the results of different experiments.

Experiment 1. At the fast flow the SF_6 and He concentration plots show the same degree of separation for the short pathways as they do at the input (Fig. 5). But for the medium and long pathways the SF_6 curve comes progressively earlier, i.e., moves to the left. For slow flow the same phenomenon is observed for all three pathways. For the medium and long pathways the curves are very similar, with the SF_6 so advanced that it is just ahead of the He, i.e., their positions have been reversed. For any given path length the SF_6 curve is more advanced at the slow flow.

Experiment 2. At the fast flow there is no difference in the separation of the SF_6 and Ar curves between the input and any of the pathways (Fig. 6). At the slow flow the SF_6 is advanced relative to the Ar by about the same amount on all pathways. One unexplained observation is that both the curves for the medium and long paths at slow flow are less spread out, i.e., they occupy a smaller

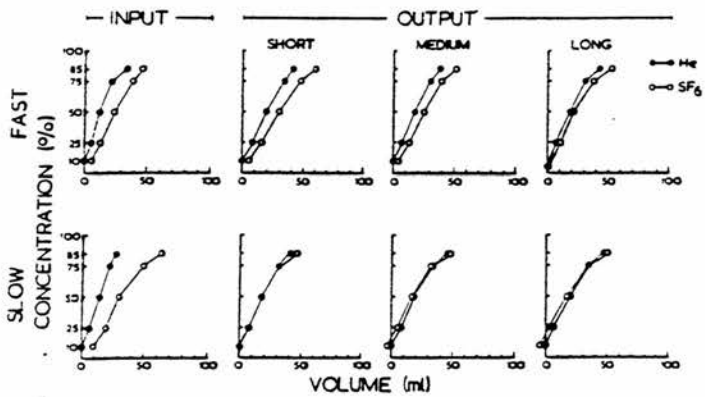

FIG. 5. Results of *expt 1*, "inspiration." Top row of four graphs shows effect of fast flow and bottom row effect of slow flow. From left to right, each row shows input concentrations, and output concentrations from ends of short, medium, and long pathways. On ordinate is plotted concentration of SF_6 and He as a percentage of those in gas mixture. On abscissa is plotted "inspired" volume with 10% He point arbitrarily defined as zero volume. Data points for SF_6 are open circles, and for He closed circles.

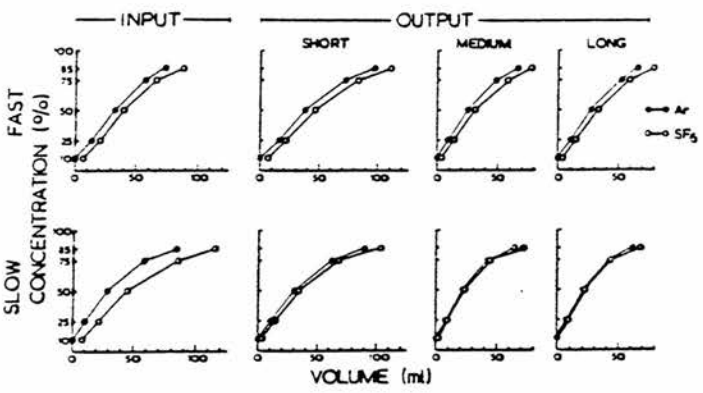

FIG. 6. Results of *expt 2*, "inspiration." Same as for Fig. 5 except that closed circles are data points for Ar.

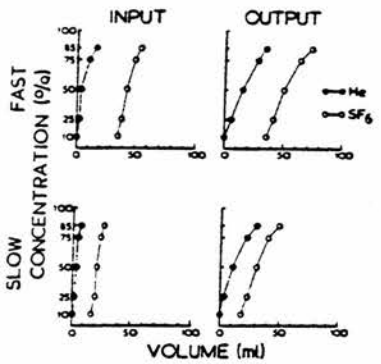

FIG. 7. Results of *expt 3*, "expiration." Same as for Fig. 5 except that there is only one input graph for each flow.

TABLE 1. *Advancement of SF_6 front relative to He and Ar fronts produced by flowing mixture containing the two gases down airway cast*

	SF_6/He	SF_6/Ar
Fast flow		
Short path	0.9	−1.3
Medium	4.2	0.8
Long	9.2	1.4
Slow flow		
Short path	16.6	15.4
Medium	18.3	15.7
Long	18.4	16.8

Measurements were made at 50% concentration points, and are expressed as inspired volume (ml).

volume than do the corresponding input curves.

Experiment 3. No difference was observed in the separation of the SF_6 and He curves between input and output at either flow during expiration (Fig. 7).

DISCUSSION

The results of *experiment 1* indicate that when a mixture of gases containing SF_6 and He flows down a branching tubular system the front of the SF_6 appears in advance of the He. In order to investigate whether this could be due to some effect dependent on viscosity *experiment 2* was performed, substituting Ar for He. The results obtained were qualitatively the same as in *experiment 1* but the degree of separation between the two gases was less. Since Ar is more viscous than He the separation should have been greater if caused by differences in viscosity.

An effect of gas density is difficult to rule out experimentally because in general density and diffusivity are closely, but inversely, related. Thus gases ranked in order of density are inversely ranked in order of diffusivity. However, we do not know of any mechanism dependent on differences in density which could explain our results.

Alternatively the explanation might lie in the differences in diffusivity of the three gases. From lowest to highest, the diffusion coefficients are ranked SF_6, Ar, He (Table 2), and it may be that some mechanism such as Taylor dispersion is enhancing the forward movement of the least diffusible gas. The difference in diffusivity between SF_6 and He is greater than that between SF_6 and Ar, and this is compatible with the greater relative advancement of SF_6 in *experiment 1* compared with that in *experiment 2*. In *experiment 3* (expiration) there was no significant separation of the SF_6 and He fronts at either flow. The shape of these fronts represents a summation of those coming from all pathways, short and long, and their resultant probably swamps any other effects. In addition, the mechanisms which are operating on the fronts during inspiration differ from those operating during expiration. Schroter and Sudlow (9) showed that four secondary vortices are generated at a junction during expiration, compared with only two on inspiration. These may have a marked mixing action, thereby diminishing any separation which might have occurred due to Taylor dispersion.

Hogg et al. (6) found that, following the inspiration of a gas mixture, more SF_6 than He entered the alveolar region of excised dog lungs insufflated with beads. They attributed this enhancement of the forward movement of SF_6 to the effects of Taylor dispersion. Thus SF_6, the less diffusive gas, would be dispersed longitudinally more then the He, and would therefore enter the alveolar region first. This phenomenon was only found after the insufflation of beads, presumably because flow went through collateral channels, distal pathways were effectively lengthened, and longitudinal diffusive mixing between gas in the alveoli and gas in the conducting airways was impeded. Without the beads longitudinal diffusive mixing is sufficient to reverse the increase in alveolar SF_6, finally giving an excess of He.

Van Liew and Mazzone (11) performed an experiment similar to ours, but used a simple straight tube. When a mixture of SF_6 and He flowed down the tube, which had initially been filled with nitrogen, the SF_6 front appeared at the far end before the He front. Their results are therefore in general agreement with ours.

How great a contribution could Taylor dispersion make to increasing the alveolar concentration of SF_6? We are unable to quantitate this precisely but some approximate calculations can be made. The first and most important point is that Taylor dispersion can only operate in that part of the tubular system being swept by the (parabolic) gas front. It can therefore only affect the transient of an inspired gas mixture, and will be inoperative once the system has been filled with the mixture. Consider now a section of tubing (Fig. 1*A*) 50% of the volume of which is occupied by the central parabolic core of a gas mixture containing SF_6 and He. The maximum separation which could occur would be if no SF_6 diffused radially while all the He rapidly distrib-

TABLE 2. *Physical characteristics of the three gases*

	SF_6	Ar	He
Diffusion coef	0.096	0.192	0.702
Viscosity	152	223	196

Diffusion coefficient measured in air ($cm^2 \cdot s^{-1}$) and viscosity at one atmosphere and 20°C (μP).

uted itself evenly across the tube. The core would then contain all the SF_6 and half the He. This would be equivalent to completely extracting the He from half the volume of the core. In practice, some SF_6 would diffuse radially so that the degree of separation would be less than this. Furthermore, a parabolic velocity profile probably never becomes fully developed in the airways, and this too would diminish the possible amount of Taylor dispersion. Put in terms of the respiratory system the equivalent volume of gas mixture separated would be something less than 25% of the volume of the conducting airways. On inspiration the first gas to reach the alveoli is SF_6 enriched, and this is followed by gas which is He enriched, containing those molecules which diffused out of the central core and were left behind in the slower moving periphery, and now being swept in. Finally, once the airways are cleared of residual gas, SF_6 and He arrive in equivalent concentrations.

The region of the airways where Taylor dispersion might be effective is discussed by Hogg et al. (6) and Wilson and Lin (13). Let L = length and r = radius of a bronchus, u = mean velocity of airflow within it, and D = diffusion coefficient of the gas. Then in airways in which $r^2u/10D\ L > 1$ radial diffusion is small compared with convective flow and Taylor dispersion is negligible. This condition operates in the upper airways. When $ru/10D \ll 1$ radial diffusion is rapid enough to obliterate the radial gradient and axial diffusion predominates, as in the respiratory bronchioles. Somewhere between, where $r^2u/10DL < 1$ but $ru/10D$ is not $\ll 1$, Taylor dispersion becomes effective. The relevant airways in the human lung were calculated by Wilson and Lin to be Weibel's (12) airway generations 8–12. With faster flow rates and gases of lower diffusivity the more peripherally situated airways would be involved, but even then it seems unlikely that the relevant region would extend down further than the terminal bronchioles. If we take 70 ml as the volume from carina to "lobular" branches (7), and 30 ml for the volume of the remaining three orders down to terminal bronchioles, this gives a total of 100 ml as the upper boundary of the volume of airways involved. 25% of this is 25 ml, so that at the very most 25 ml of a mixture could be separated into its component gases. Weibel's generations 8–12 have a volume of 33.2 ml, and 25% of this is 8.3 ml, which is perhaps a more reasonable estimate of the volume which could be separated by the human lung. The maximum separation obtained in our experiment with the pig lung cast was 18.4 ml using one lung equivalent to about 37 ml for both lungs. This is approximately four times the calculated value for the human lung.

These calculations indicate that the separation of SF_6 should be more marked on the longer pathways because of the greater volume of airways between branches of given diameters. Increasing the flow rate, i.e., the value of u, should move the effective region downwards, and in our experiment, in which the cast is cut off peripherally, the effect should be decreased. Finally, the greater the difference in diffusivity of the two gases the greater the separation. Thus an He/SF_6 mixture would be more separated than an Ar/SF_6 mixture. These three effects were each observed in our experiment (see Table 1) and it is likely that the explanation for them is Taylor dispersion.

What part, if any, does Taylor dispersion play in the physiology of normal respiration? The three gases commonly breathed, oxygen, carbon dioxide, and nitrogen, have diffusion coefficients which differ only slightly from each other (0.20, 0.15, 0.22 $cm^2 \cdot s^{-1}$ respectively). Furthermore, their differences in concentration between dead space gas and air are not large, so that the absolute volume of these gases which could be separated by Taylor dispersion would be minute. It therefore seems unlikely to play any role in normal breathing, such as in the generation of the slope of the alveolar plateau. Chang (2) came to the same conclusion on the basis of calculations using a convective-diffusion equation.

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Publication 23

Davies,A. & Kohl,J.(1979)

Interaction of lung stretch and irritant receptors in determination of duration of expiration and subsequent inspiration.

Proc. Swiss Soc. of Exp. Biol. April,1979.

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Interaction of lung stretch and irritant receptors in determination of duration of expiration and subsequent inspiration.

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The respiratory response of anaesthetised rabbits to injections or inhalation of aerosols of histamine is of two types. Shortening of expiratory duration (t_E) can precede shortening of inspiratory duration (t_I) by a few breaths and then they both shorten together or t_I could remain constant while t_E shortens until an augmented breath preceded a large reduction in t_I . We call these two types of accelerated breathing continuous and discontinuous respectively. These patterns were mutually exclusive and were not modified by block of pulmonary stretch receptors. The response to histamine was abolished by vagotomy, we therefore attribute these differences in pattern to the activity of rapidly adapting receptors.

Publication 24.

Davies,A. Sant'Ambrogio,F.& Sant'Ambrogio, G.(1979)

The initiation of inspiration.

J. Physiol. 295, 41-42p.

The initiation of inspiration

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It is generally accepted that the activity of pulmonary stretch receptors inhibits inspiration. In artificially ventilated animals this relegates inspiratory efforts to periods of lung deflation. If stretch receptor activity were the only determinant of inspiratory timing, removing this activity should result in a pattern of inspiratory efforts which is identical to that after vagotomy. We have investigated other influences on the timing of inspiration.

New Zealand White rabbits were anaesthetized with 30 mg/kg sodium pentobarbitone. A tracheal cannula was inserted and the pattern of breathing was recorded using a pneumotachograph whose output was electronically integrated to provide a record of tidal volume. The activity of the cut end of a root of the right phrenic nerve was simultaneously recorded. The animals were paralysed with gallamine triethiodide (Flaxedil; May & Baker). Pentobarbitone sodium was administered at the same rate as before paralysis to maintain anaesthesia. Ventilation was by intermittent positive pressure in a pattern which closely followed spontaneous breathing. Lung stretch receptors were paralysed by ventilating the animal with 200 p.p.m. SO_2 in air (Callanan, Dixon & Widdicombe, 1975; Davies, Dixon, Callanan, Huszczuk, Widdicombe & Wise, 1978). Abolition of the Hering-Breuer inflation reflex was used as an index of stretch receptor paralysis. The presence of irritant receptor activity was demonstrated by rapid deflations of the lungs which caused an increase in the frequency of the bursts of phrenic discharge (Widdicombe, 1954).

With stretch receptors functioning phrenic discharge invariably occurred during the deflation phase of ventilation. When stretch receptors were blocked phrenic discharge occurred with no set phase relation to ventilation if tidal volume was below resting spontaneous tidal volume. Phrenic discharge was synchronous with the inspiratory and expiratory phases of the pump at higher tidal volumes. Stopping the pump in its deflation phase immediately reduced the frequency of phrenic bursts. Bilateral vagotomy produced a pattern identical with that seen with stretch receptor block and low ventilation volumes.

We have demonstrated a vagally mediated inspiratory initiating effect. Since J-receptors are reported to be inactive under our conditions (Paintal, 1973; Guz & Trenchard, 1971) this probably originates from rapidly adapting lung irritant receptors.

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Publication 25.

Horsfield ,K., Davies,A. & Cumming,G.(1980)

Effect of flow oscillations on stationary concentration fronts in a hollow cast of the airways.

Lung 157, 103-111.

Lung 157, 103–111 (1980)

Lung

Effect of Flow Oscillations on the Stationary Concentration Front in a Hollow Cast of the Airways

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Abstract. Nitrogen was blown down a hollow cast of a pig lung at a steady flow of 6.7 ml/s and the concentration of oxygen and nitrogen were measured at various points through small holes in the cast, using a mass spectrometer. A stationary concentration front between nitrogen and oxygen was found within the cast. When an oscillation was imposed on the flow by a reciprocating pump the concentration front moved up the cast, and this movement was more marked at higher pump frequencies and stroke volumes. This suggests that series dead space should diminish with both increasing frequency and increasing stroke volume of the heart.

Key words: Airway cast – Diffusive mixing – Dead space – Stationary front – Oscillatory flow

Introduction

The problem of how gas mixes in the lungs, and especially the part played by molecular diffusion in this process, has been the subject of considerable debate and controversy [1, 2, 8, 9, 10]. Analysis of a model by Cumming et al. [2] suggested that when inspiratory flow and molecular diffusion of a gas occur in opposite directions the two processes come into balance. Thus if a breath of 100 per cent oxygen is taken following air breathing the nitrogen in the residual gas diffuses outwards during inspiration and convective flow simultaneously washes it back in. At some point (250 ml down the airways in the model analysed) a front of nitrogen concentration develops which is stationary if the flow is constant. When inspiratory flow is increased this front becomes established further down the airway, and when it is decreased it becomes established more proximally. These predictions of the model analyses have been confirmed by more sophisticated analyses of different lung models by Paiva et al. [8, 9] and demonstrated experimentally by Engel et al. [4, 5] and Fukuchi et al. [6] working with dog lungs both in situ and excised. Fukuchi et al. showed

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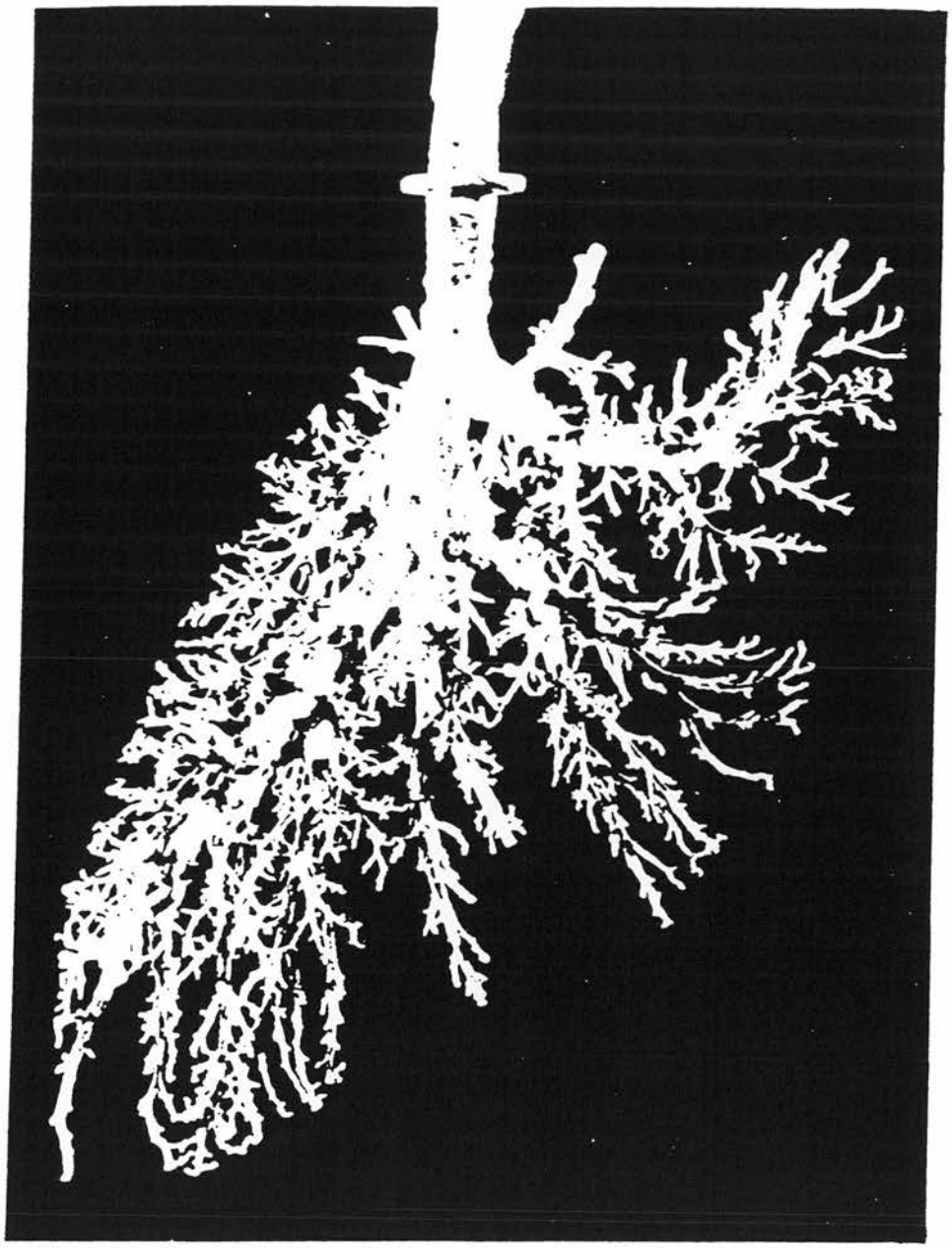

Fig. 1. Hollow cast of a pig lung. Hole 11 is situated on the branch at the extreme lower left of the figure.

that the rate of gas mixing in the airways is materially affected by the cardiogenic pulsations, which they suggested have an effect equivalent to an increase in diffusion coefficient by a factor of five.

The ability of a stationary concentration front to form during steady flow depends on the anatomy of the airways, in which total cross-sectional area increases with distance from the carina. Mean flow velocity at any point is inversely proportional, and molecular diffusion directly proportional to cross-sectional area, so that with respect to changing distance from the carina one process diminishes while the other increases [7]. A balance point is therefore likely to exist. Of course oxygen continues to enter the alveoli during inspiration, moving down by convective flow and molecular diffusion, so that the mean concentration of nitrogen in the alveolar region steadily falls. This, however, has little effect on the position of the front [2, 8].

The purpose of the present study was to investigate the behaviour of the stationary interface in a hollow cast of a bronchial tree, with particular regard to the effect of oscillation in the flow mimicking cardiogenic mixing.

Methods

A positive (hollow) cast of the bronchial tree of one pig lung was used for the study (Fig. 1). It was made as described by Davies [3]. Briefly, a negative cast was made from wax, and the tissue macerated with concentrated hydrochlorid acid. The cast was largely, though not entirely, complete down to the branches of about 1 mm diameter. It was coated with colloidal silver, except for the tips of the end branches, and then electroplated in silver. Finally, the wax was removed from the silver cast by melting and blowing hot air through.

The general principle of the present experiment was to pass nitrogen down the cast at a sufficiently steady flow that a front might become established between it and atmospheric oxygen diffusing up through the open ends of the cast. The concentration of nitrogen and oxygen at a given point was sampled through a hole drilled in the wall of the cast. Eleven such holes of 0.8 mm diameter were drilled at intervals of 10 to 20 mm on a long bronchial pathway, starting with the main bronchus. The drilling was done with care using a finger-held pin chuck, an instrument which holds the drill bit and which has a pen grip to enable it to be rotated by moving the fingers to and fro. This was done in order to minimize the risk of burring the inside of the hole. The internal diameter at the site of each hole was measured using a needle with a small hook on the point, produced by bending the tip at right angles. This needle was inserted into the hole up to the far wall of the tube and a mark was made on its shaft where it entered the hole. It was then withdrawn until the hook caught the internal edge of the hole and another mark made. The distance between the two marks on the needle represents the internal diameter of the branch minus the thickness of the hook, which was 0.2 mm, so this amount was added on to each measurement. The distance between each hole was measured and summed to give the path length from hole 1 to each of the other holes. The data obtained from the cast are shown in Table 1.

Gas concentrations at each site were measured using the blood gas inlet of a Centronic 200 MGA mass spectrometer. This instrument is designed to work with

very low sample flows, and will switch off automatically if the flow rises above about 10^{-4} ml/s. It was chosen for the experiment in order to be able to sample gas within the cast without producing significant secondary flows, thereby disturbing the interface. A special sample probe tip had to be made for the purpose. About 30 mm of plastic tubing, 0.8 mm o.d. was attached to the flexible tubular inlet sample line. A few millimeters of soft wire, fitting tightly into the lumen of the plastic tube, was pushed into the tip so that it became completely obstructed. Using a scalpel blade, successive portions of the tip, both tube and soft wire, were cut off until sampling at the desired rate became possible. This occurred when about 1 mm of soft wire remained, permitting a molecular leak round it. Finally, the remaining wire was pushed up the tube for about 1 mm allowing the tip to fit snugly into a hole in the cast, but being too wide to pass through it (Fig. 2). The response time of this system was about 1 minute, so that transients of concentration could not be measured.

Nitrogen was chosen as the "inspired" gas in preferance to oxygen because 100 per cent oxygen adversely affects the mass spectrometer filament. Thus there was a reversal of the usual physiological situation in which oxygen is inspired and alveolar nitrogen diffuses up the airways.

Before and after each set of measurements the scale readings for zero nitrogen and oxygen were obtained by sampling 100% argon, and atmospheric nitrogen and oxygen by sampling air.

Measurement of Stationary Interface

Preliminary experiments were conducted in order to find out what flow of nitrogen was required to establish a stationary interface. This was most satisfactorily achieved at 400 ml/min (6.7 ml/s), faster flows pushing the front out of the distal ends of the cast, but slower flows not producing very much change in its position.

Nitrogen passed from a high pressure cylinder through a reduction valve attached to the cast (main bronchus) by rubber tubing. Fine regulation of flow was achieved by means of a screw clip on the tubing, which was pierced by an intramus-

Table 1. Morphometric data for the cast at each hole

Hole	Internal diameter (cm)	Path length from hole 1 (cm)
1	1.08	0.0
2	1.20	1.55
3	1.02	3.85
4	0.78	6.10
5	–	7.90
6	0.60	10.05
7	0.48	11.95
8	0.38	13.00
9	0.32	14.75
10	0.24	16.65
11	0.14	17.55

Diameter at hole 5 could not be measured because it was opposite a bifurcation

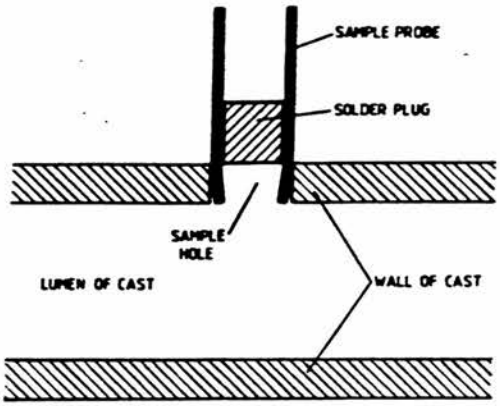

Fig. 2. Cross-section of the sample line tip placed in a hole in the cast wall.

cular injection needle left in situ proximal to the screw clip. This leak helped to stabilize flow, probably by permitting a higher pressure of gas in the tubing. Flow was measured by water displacement in an inverted graduated cylinder timed by a stop watch.

Flow was established at 6.7 ml/s and the system allowed to stabilize for two minutes; the gas concentrations were then measured at each hole in turn. The holes were each covered with a small piece of tacky material, which was removed only from the hole at which measurement was being made. The mass spectrometer sample probe was inserted gently into the hole and wedged, and sufficient time allowed to elapse for a steady reading to be obtained. Hole 4 repeatedly gave unsteady readings, probably because of a slight misfit with the sample probe, so measurements were not taken at that hole.

Effect of Oscillations in the Flow

The method was similar to that used with steady flow. A pump of variable stroke volume and frequency was attached to a side arm of the tubing connecting to the cast, so that an oscillation could be imposed on the steady flow delivered from the gas cylinder. Nine measurements of the interface position were made with stroke volume of 3.0 ml, 5.5 ml, and 8.0 ml, each at 1, 2, and 3 Hz. Because of the long response time the transients of concentration could not be measured, the value obtained being a mean concentration.

Results

Figure 3 shows the concentration of oxygen in the cast, both for steady and oscillatory flow. With steady flow low concentrations of oxygen are detectable as far up as hole 2, but the rising concentration of the front is situated at holes 9, 10, and 11, reaching a maximum of 3.0 per cent. Between holes 2 and 8 there is a slightly irregular plateau of concentration. As frequency and stroke volume of the oscillations increase, the front moves to the left (up the cast) and the concentration of oxygen rises, both at the front and at the plateau. Finally, the plateau is obliterated as it is overtaken by the advancing front.

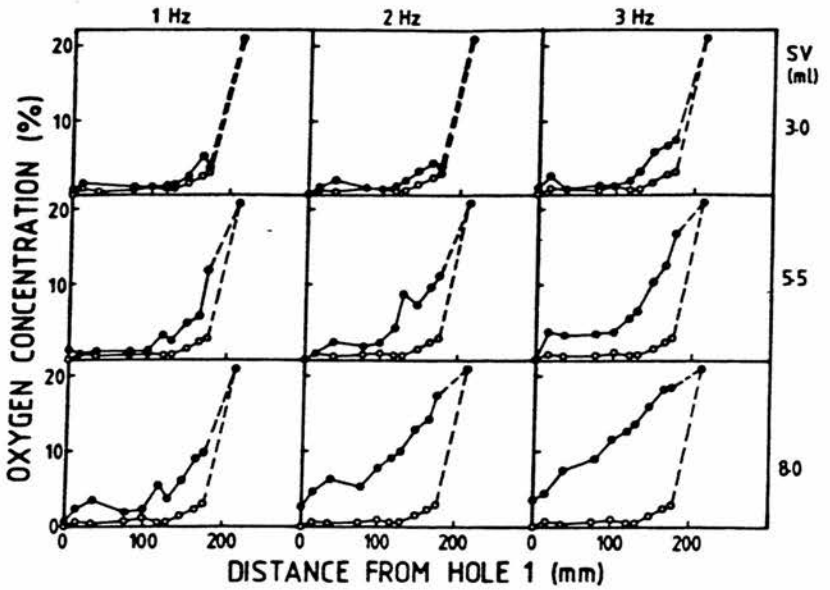

Fig. 3. Effect of oscillations of the flow on the concentration profile of oxygen. Abscissa-distance down cast from hole 1. Ordinate-oxygen concentration. Data for hole 4 excluded. Open circles show the front when there is a constant flow of 6.7 ml/s, and this is repeated on each panel to facilitate comparison. Closed circles show the effect of superimposing oscillations on the flow at 1, 2, and 3 Hz, with stroke volumes (SV) of 3.0, 5.5, and 8.0 ml. The point at 215 mm and 21 per cent concentration is not a data point. It represents the end of the most distal branch of the cast, 40 mm from hole 11, at atmospheric oxygen concentration. It is joined to the data points for hole 11 by dashed lines.

Discussion

Technical Errors

The main problem with the measurement of concentration is whether the mass spectrometer sample flow is sufficient to upset the flow regime in the cast, and in particular whether it might result in air being actively sucked up the airways. There were about 200 branches on the cast of a diameter approximately equal to that in which hole 11 was situated. At a rough estimate flow in this branch would be about 1/200 that at hole 1, that is about 0.033 ml/s. The spectrometer sample rate was 10^{-4} ml/s or less, less than 1/30 of the flow at hole 11. This is probably sufficiently low not to cause any significant effect on the flow in the cast. As shown in Fig. 2 the tip of the sample probe did not project into the lumen of the cast, and therefore did not perturb flow on that account. The concentration fronts proved to be stable and reproducible; they could be obtained a second time after a period of 15 minutes, and could be obtained on subsequent occasions after the apparatus had been dismantled and reassembled.

The distribution of flow between the various branches of the cast was unlikely to be the same as in the corresponding airways during life. This is because in the open

ended cast flow is determined by the resistance of each pathway, whereas in the lung the expansion of the alveolar region is an important determinant of flow. Nevertheless, the cast is a branching tubular tree of increasing total cross-section peripherally which has the same morphology as the airways. Although the detailed shape of the front may have been slightly affected by the flow distribution it is unlikely that the general results were materially altered.

Shape and Position of the Concentration Front

The general use of symmetrical models for diffusion calculations results in the finding of identical concentrations in airways of any given generation or diameter. In real lungs the bronchial tree is markedly asymmetrical with short pathways coming off large airways, so that nitrogen can reach these larger airways by diffusion up the short pathways much more easily than symmetrical model analysis would suggest. Thus alveolar gas may reach higher up the tree than expected. The form of the front must be determined partly by this asymmetry, and not just by diffusion up a trumpet-shaped axisymmetric tube. This probably explains the plateau of oxygen concentration in the cast.

Paiva et al. [9] calculated that the centre of the stationary front (strictly, its inflection point) is situated at a point in the airways such that $\dot{V}/D = dA/dx$, where $\dot{V}$ = tracheal flow, D = diffusion coefficient, and dA/dx = change in total cross-sectional area with respect to change in linear distance in the airways. In this experiment $\dot{V}$ = 6.7 ml/s, D = 0.25 cm²/s, so $\dot{V}/D = 27$. Thus the front should become established at the point where the summed cross-sectional area is increasing by 27 cm² per cm distance along the airways. Since atmospheric oxygen represents a fractional concentration of 1.0, 10.5 per cent represents a fractional concentration of 0.5. But only 3 per cent was observed, so that the centre of the front was beyond hole 11, and only its upper end was observed. During inspiration in life flow is greater than 6.7 ml/s in one lung in the pig and the front is therefore established further down the airways where summed cross-sectional area increases more rapidly with distance. It is because the cast ended at branches of about 1.0 mm in diameter that the flow had to be used. At slower flows the front should have been established more proximally, but this was probably not possible because of the slow increase of cross-sectional area with distance in the large airways. Indeed, in the large airways cross-sectional area actually decreases with distance [11].

Effect of Oscillations

The oscillations seen on records of gas concentration obtained within the airways is attributed to periodic reversal of flow during cardiac contraction [12]. In order to investigate the effects of pulsatile flow on gas mixing in the cast it was essential to choose a range of stroke volume and frequency which would produce flow reversal. Considering a stroke volume of 3 ml at 1 Hz, the average flow due to the pump for one half cycle would be 3 ml in 0.5 s, or 6 ml/s. In one half of the cycle this would nearly halt flow down the cast with the inlet flow at 6.7 ml/s, and in the other half of the cycle it would nearly double the flow. With a stroke volume of 3 ml at 2 Hz, or 5.5 ml at 1 Hz, pump flow would be 12 or 11 ml/s, against 6.7 ml/s at the inlet to the cast. This should be sufficient to cause reversal of flow in one half of the cycle.

With oscillations at the two higher stroke volumes the 0.5 fractional concentration point of the front became established at or proximal to hole 11. Other things being equal, such as time, diffusivity, and anatomy, the anatomical dead space is determined by the position of the front. Thus in the cast dead space appeared to be reduced by the oscillations, confirming the findings of Engel et al. [4] and Fukuchi et al. [6] that dead space is reduced by the action of the heart. The cast experiment also suggests the possibility that the reduction in dead space might be greater with increasing stroke volume. In an anaesthetised animal, if heart rate goes up stroke volume will usually fall, the two effects tending to cancel each other and thus not be easily detected.

Gas Mixing in the Lung

The airways constitute one continuous system, from the mouth to the alveoli, and if gas mixing within it were complete the concentrations at the mouth would be the same as those in the alveoli. Nevertheless, although it is one system, it is common practice in physiology to divide the airways into two gas mixing zones, the anatomical or series dead space, broadly corresponding to the conducting airways, and the alveolar region distal to the terminal bronchioles. The demonstration of the existence of a stationary front during inspiration made possible a functional definition of the dividing line between the two, series dead space being proximal to the front and the alveolar zone distal to it. Gas mixing takes place at the front, diminishing series dead space as it progresses, and distal to the front, reducing alveolar inhomogeneity as it progresses. Both cardiac oscillations and the use of gases of higher diffusivity result in the front being established higher up the airways, and during breathholding it moves further up. Engel et al. [4] showed in dogs that dead space rises by 13 per cent with cessation of heartbeat, a mean rise of 15 ml from 113 ml. If this is considered to be equivalent to an increase of say 20 ml in man, then with an FRC of 3000 ml and a tidal volume of 500 ml, alveolar ventilation would be about 5 per cent less without cardiogenic mixing in the conducting airways. Distal to the front gas mixing progresses with time due to molecular diffusion, and is thus more rapid with more diffusable gases. It must be pointed out, however, that while the cast experiment and the work of Engel et al. [4] have both shown that oscillations increase mixing in the conducting airways, in neither case was the effect on alveolar mixing investigated. Thus at present there is no evidence in the literature as to whether or not cardiac oscillations increase alveolar mixing.

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Publication 26.

Davies,A.,Vizek,M. & Widdicombe,J.G.(1980)

Effect of brief mechanical stimulation of the larynx on pattern of breathing in the rabbit.

In: Proc. International Union of Physiological Sciences (Budapest) 1980.

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THE INFLUENCE OF BRIEF MECHANICAL STIMULATIONS OF THE LARYNX ON PHRENIC DISCHARGE. A. DAVIES, M. VIZEK, J.G. WIDDICOMBE. Dept. of Physiology, St. George's Hospital Medical School, Tooting, London, SW17 ORE, U.K.

In pentobarbitone-anaesthetized rabbits we isolated the larynx from the trachea and pharynx and subjected it to brief (100 ms) positive and negative pressure pulses (5-41 cm H_2O). When given in inspiration these pulses caused a short inhibition of phrenic discharge but prolonged the total inspiratory duration. Pressure pulses in expiration shortened its duration. No expiratory muscle activity was stimulated by pulses in either phase. The detailed patterns of these changes and their underlying receptor mechanisms have been studied. The possibility of using this preparation to study airway hyper-reactivity will be discussed.

Publication 27.

Hanacek,J.,Davies,A.,Widdicombe,J.G.&
Korpas,K.(1980).

Stretch receptors of the lung and their effects on lung defensive reflexes.

In: Proc. International Union of Physiological Sciences (Budapest)

2681

STRETCH RECEPTORS OF THE LUNG AND THEIR EFFECT ON LUNG DEFENSIVE REFLEXES.

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The authors observed changes in the intensity of the expiratory reflex and cough in anaesthetised rabbits in which lung stretch receptors were blocked by SO_2 and stimulated by inflation of the lungs. Cough was induced by NH_3 on the larynx. During stretch receptor block it was significantly reduced in comparison to the control. Lung inflations by 0.5; 1.0 and 1.5 kPa have significantly increased the intensity of the expiration reflex elicited by mechanical stimulation of the vocal folds. The authors suggest rabbits lung stretch receptors are part of the lung defensive reflexes regulation.

Publication 28.

Davies,A.,Sant'Ambrogio,F. & Sant'AmbrogioG.(1980)

Control of postural changes of end expiratory volume by airways slowly adapting mechanoreceptors.

Respir. Physiol. 41, 211-216.

CONTROL OF POSTURAL CHANGES OF END EXPIRATORY VOLUME (FRC) BY AIRWAYS SLOWLY ADAPTING MECHANORECEPTORS

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Abstract. We recorded the e.m.g. activity of the diaphragm and of an abdominal muscle (ext. oblique) and the respiratory volume in anesthetized rabbits challenged with head-up tilting and positive pressure breathing (PPB). Both maneuvers determined an inhibition of inspiratory activity and an activation of abdominal muscles, the latter being especially marked with tilting. After cervical vagotomy neither the inspiratory inhibition nor the abdominal recruitment was present during tilting and PPB and the FRC increase was more pronounced. Sulphur dioxide was given in the inspired air (200 ppm) to selectively block the slowly adapting mechanoreceptors. Such blockade was indicated by the absence of the Hering-Breuer inflation reflex. The permanence of other respiratory reflexes was shown by a parodoxical response to inflation and by a still evident response to deflation. With SO_2 block, both tilting and PPB did not elicit either the inspiratory inhibition or the abdominal muscles activation, leading to an FRC shift similar to that observed after vagotomy. We conclude that the slowly adapting mechanoreceptors subserve a reflex mechanism relevant in controlling FRC.

F.R.C.
Posture
Stretch receptors
Tilting
Vagal reflexes

The abdomen and thorax are mechanically interdependent and during postural movements there are marked changes in volume between these two compartments which are mainly due to gravitational forces acting on the abdominal contents. Powerful reflexes have been found which activate the abdominal muscles when lung volume is increased (Bishop, 1964). The afferent pathways of these reflexes run through the vagus nerves, but the identity of the receptors involved has not been definitely clarified, though experiments in which the vagal conduction was partially blocked indicated a possible role of the slowly adapting stretch receptors (Mortola and Sant'Ambrogio, 1973).

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This study is aimed at identifying these receptors using a recent technique for selectively blocking slowly adapting stretch receptors in the airways (Davies *et al.*, 1978).

Methods

The experiments were performed on 10 New Zealand white rabbits weighing between 2.0 and 2.5 kg. They were anaesthetized with sodium pentobarbital injected into the marginal vein of the ear. The trachea was cannulated 1 cm below the larynx and a catheter inserted in a femoral vein for further injection of anaesthetic.

Respiratory airflow was measured by a Fleisch 00 pneumotachograph connected to the tracheal cannula. Tidal volume was obtained by electronic integration of the flow signal. The electrical activity of the external abdominal oblique muscle was recorded by two thin wire hooks inserted in the muscle layer of the lateral abdomen and that of the diaphragm by similar thin wires inserted into its sternal portion through a small incision in the abdominal wall. The four signals were recorded on a Brush-Gould pen oscillograph.

In each experiment the animal was exposed to both positive pressure breathing (P.P.B.) and head-up tilting. The pressure was adjusted to produce an increase in end-expiratory volume similar to that obtained by tilting. This was usually about 8 cm H_2O. The pressure was applied by connecting the trachea to a large drum maintained at the required pressure by a pump and pressure regulator. The rabbit was strapped to a wooden board and tilted by lifting the head end from the horizontal to vertical position.

Sulphur dioxide, used to block airways stretch receptors, was prepared and administered by the method of Davies *et al.* (1978). Block was considered complete when the Breuer-Hering reflex was absent on lung inflation at +15 cm H_2O. An increase in respiratory frequency on P.P.B. and the presence of a deflation reflex indicated the activation of rapidly adapting receptors in the airways. In most experiments 10 min of SO_2 inhalation led to complete abolition of the Breuer-Hering reflex, increased T_I, V_T, and decreased T_E (Davies *et al.*, 1978).

The experimental procedure was as follows. The Breuer-Hering inflation reflex to +15 cm H_2O was measured as the ratio of the first expiratory time during lung inflation to control expiratory time (inhibitory ratio: I.R.). A series of P.P.B. (+8 cm H_2O) and head-up tilting were carried out with time allowed between maneuvers for breathing to return to control pattern. The SO_2-air mixture was administered and the Breuer-Hering reflex re-tested at intervals. When the reflex was completely abolished, as indicated by an I.R. $\leqslant$ 1.0, the animal was subjected to a further series of P.P.B. and tilting. The Breuer-Hering reflex began to reappear 20–30 min after the end of SO_2 exposure but never fully recovered its control value.

The animals were then bilaterally vagotomized in the neck, after applying 2% lignocain to the vagi, and the P.P.B. and tilts repeated.

The accuracy of the measurements of the increase in FRC with tilting and P.P.B. was checked connecting the pneumotachograph to a 100-ml glass syringe which could be tilted (outlet up) or connected to a positive pressure source. Its piston was supported by a weak spring and the corresponding volume displacements read on the graduated syringe and recorded as the analog output from the integrator.

Results and discussion

Ten rabbits were subjected to several periods of head-up tilting and P.P.B. Figure 1 shows the response of a typical rabbit to P.P.B., on the left, and head-up tilting, on the right, with control conditions (top), during stretch receptors block (middle) and after bilateral vagotomy (bottom). Both P.P.B. and tilting caused an increase in Functional Residual Capacity (F.R.C.) with an arrest of inspiratory activity and an activation of the external oblique muscle. This latter effect was

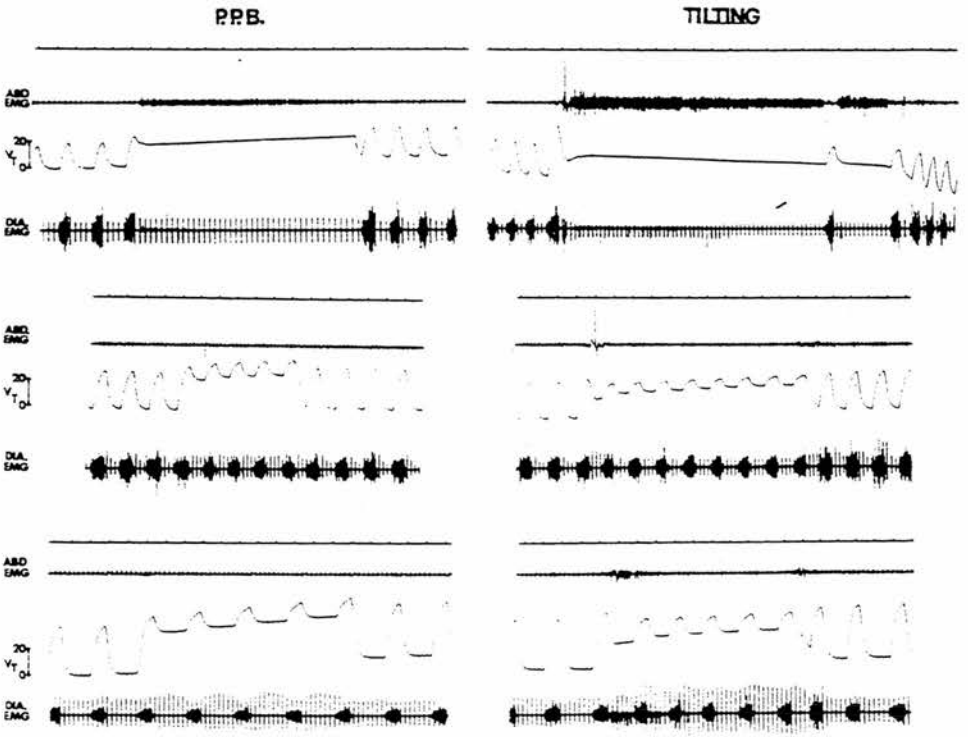

Fig. 1. Anaesthetized rabbit. Effect of positive pressure breathing (P.P.B.; tracings on the left) and head-up tilting (tracings on the right) on the activity of the external oblique m. (ABD. emg), the diaphragm (DIA. emg) and the tidal volume (V_T) in control condition (top tracings), after SO_2 inhalation (middle tracings) and post-vagotomy (bottom tracings). Time marker = 1 sec.

TABLE I

Increase in end-expiratory volume (F.R.C.) with head-up tilting in control condition, after SO_2 inhalation and post-vagotomy. Each number represents the mean of 3–5 trials ± SE. A two-tailed *t*-test was performed to evaluate the significance of the difference. Two asterisks indicate a highly significant difference with the corresponding control value ($P < 0.01$). One asterisk a significant difference ($P < 0.05$). No statistical difference was ever detected between 'SO_2' and 'post-vagotomy'. In rabbit #4 the differences between either 'SO_2' or 'post-vagotomy' and control were not statistically significant ($0.05 < P < 0.1$). Rabbit #10 died after vagotomy

Rabbit No.	Control (Δ FRC, ml)	SO_2 (Δ FRC, ml)	Post-vagotomy (Δ FRC, ml)
1	16.8 ± 0.75	23.8 ± 0.77**	22.3 ± 0.62**
2	24.6 ± 0.35	35.0 ± 0.70**	29.3 ± 0.70**
3	20.0 ± 0.95	36.0 ± 0.64**	36.8 ± 1.01**
4	19.7 ± 1.42	22.9 ± 0.00	23.4 ± 1.23
5	12.3 ± 0.44	18.6 ± 1.17**	17.6 ± 0.82**
6	17.6 ± 1.20	29.7 ± 0.48**	27.4 ± 0.88**
7	10.2 ± 0.25	29.7 ± 0.57**	29.7 ± 0.63**
8	19.3 ± 0.78	24.3 ± 1.09**	26.2 ± 1.25**
9	12.9 ± 1.13	22.3 ± 0.42**	24.1 ± 0.49**
10	12.6 ± 0.78	23.4 ± 1.40**	–

more marked as a result of tilting, despite a similar shift in F.R.C. (compare the top two tracings).

In each of the ten rabbits studied inspiratory inhibition and abdominal activation due to P.P.B. and tilting were abolished by bilateral vagotomy (fig. 1, bottom records) and the increase in F.R.C. became significantly greater, with the exception of rabbit #4 which, however, showed a similar trend (fig. 1, bottom records and table 1). The respiratory responses to head-up tilting and their reflex nature with a vagal afferent pathway had been described in the past and their essential similarity with the Breuer-Hering inflation response appropriately recognized (Gordh, 1945; Moruzzi, 1945).

Sulphur dioxide inhalation, with a total disappearance of the inflation apnea, led to essentially similar results to vagotomy. Specifically an equal increase in F.R.C. with tilting (fig. 1, record in the middle of right panel and table 1).

Absence of abdominal muscle activity can explain the greater increase in end-expiratory volume on tilting and P.P.B. during SO_2 blockade and after vagotomy (fig. 1 and table 1). No significant increases in lung compliance, which could possibly contribute to a greater shift in F.R.C., are known to occur after vagotomy or vagal block in rabbits (Karczewski and Widdicombe, 1969).

The finding that the SO_2 blockade of slowly adapting stretch receptors and vagotomy led to similar responses seems to indicate that the afferent pathway responsible for the abdominal muscles activation is uniquely originating from these endings. Similar conclusions had been reached by Mortola and Sant'Ambrogio

(1973) in experiments in which the vagal conduction was partially blocked with current of increasing strength. The present evidence is based on a better documented selectivity of stretch receptors blockade and therefore more convincing.

The decrease in tidal volume produced by P.P.B. and tilting can be accounted for by the altered circumstances of contraction of the inspiratory muscles dependent on their tension-length diagram (Agostoni, 1964; Marshall, 1962; Sant'Ambrogio and Saibene, 1970).

Under control conditions tilting produced a considerably greater activation of the abdominal muscles than P.P.B. (fig. 1, compare the top two records). This difference cannot be explained in terms of different levels of stretch receptors activity because the increase in F.R.C. was similar. The greater activity in the external oblique muscle may be interpreted as being introduced by an additional proprioceptive input from abdominal muscles stretched by the weight of the abdominal viscera. This proprioceptive input seems to be incapable of activating the abdominal muscles by itself: in fact tilting did not produce any effect after SO_2 blockade and vagotomy. It may however, have a considerable facilitatory influence on the vagally mediated excitatory input originating from the stretch receptors in the airways. Similarly Bishop (1964) demonstrated the importance of these facilitatory influences from the passively stretched abdominal muscles during positive pressure breathing.

Our experiments demonstrate a role of airways slowly adapting receptors in the reflex regulation of end expiratory volume which is profoundly influenced by postural changes. They contribute to a homeostatic mechanism which minimizes the changes in Functional Residual Capacity introduced by the gravitational influences of posture.

In un-anesthetized rabbits vagal reflexes are relatively weaker than with pentobarbital anesthesia (Sant'Ambrogio and Widdicombe, 1965) and head-up tilting causes a less marked slowing of breathing (Moruzzi, 1945). In this condition an activation of the abdominal musculature might depend to a greater extent on the proprioceptive influences originating from the muscles themselves and on conscious reactions.

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ONSET OF INSPIRATION IN RABBITS DURING ARTIFICIAL VENTILATION

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SUMMARY

1. We have investigated whether pulmonary stretch receptors are the only lung receptors determining the time of onset of inspiratory efforts in anaesthetized, paralysed rabbits.

2. New Zealand White rabbits were anaesthetized, paralysed and ventilated by intermittent positive pressure with a pattern that closely followed spontaneous breathing. Inspiratory efforts were recorded as bursts of activity in a root of the phrenic nerve. Lung stretch receptors were blocked with SO_2 in air. Abolition of the Breuer-Hering reflex was used as an index of stretch receptor block.

3. With stretch receptors functioning phrenic discharge invariably occurred during the deflation phase of ventilation. With stretch receptors blocked phrenic discharge occurred with no set relation to ventilation at spontaneous resting tidal volume but was locked to inflation and deflation phases of lung volume at 30% higher tidal volumes.

4. Bilateral vagotomy produced a pattern of phrenic discharge identical with that seen with stretch receptor block and low ventilating volumes.

5. Thus we have demonstrated a vagally mediated inspiratory initiating effect; it probably originates from rapidly adapting lung irritant receptors.

INTRODUCTION

Activity from rapidly adapting lung (irritant) receptors has been recorded as early as 1929 (Keller & Loeser, 1929). The intra-epithelial nerve endings which are the probable origin of this activity had been described even earlier (Larsell, 1921; Elftman, 1943). While investigators have been willing to accept that this activity may contribute to patterns of breathing seen in pathological states (Mills, Sellick & Widdicombe, 1969) and in part be responsible for gasps and sighs (Knowlton & Larrabee, 1946; Reynolds, 1962; Sellick & Widdicombe, 1970), information on their influence in normal quiet breathing has not been forthcoming. We have demonstrated that brief pulses of lung inflation or deflation (which intensely stimulated irritant receptors) given in expiration shortened this phase of the breathing cycle (Davies,

1978). Identical pulses given in inspiration provoked an augmented breath (Davies & Roumy, 1978), which supports an inspiratory augmenting role for irritant receptor activity. The present experiments were undertaken to investigate the influence of irritant receptors on the onset of inspiration in eupnoeic breathing.

METHODS

Experiments were conducted on 8 New Zealand White rabbits weighing from 2 to 2·5 kg. They were anaesthetized with an I.V. injection of sodium pentobarbitone. 30 mg/kg, and a tracheal cannula and femoral venous cathether were inserted. The animal was placed supine and the electrical activity of the central cut end of one of the roots of the right phrenic nerve, placed under liquid paraffin, was recorded. Airflow was measured by a Fleisch pneumotachograph head connected to the tracheal cannula. Tidal volume was obtained by integrating flow electronically. Air flow, tidal volume, the electroneurogram and its continuous time integral were displayed on a Brush–Gould pen recorder. Lung stretch receptors were blocked by causing the animals to breath 200–400 parts per million SO_2 (Davies, Dixon, Callanan, Huszczuk, Widdicombe & Wise, 1978). The gas mixture was prepared in a Douglas Bag and drawn through a T piece attached to the tracheal cannula using a water pump. The animals were paralysed with gallamine triethiodide and ventilated by a Harvard pump with a tidal volume and frequency identical to their spontaneous breathing. End-tidal CO_2 was monitored by an infra-red gas analyser (Beckman L.B.1) and remained within 0·5 % of the spontaneous breathing level. In paralysed animals supplementary doses of anaesthetic were administered at the same rate as before paralysis.

Before blocking lung stretch receptors positive pressure breathing (+5 and +10 cm H_2O) produced the Breuer–Hering inflation reflex, negative pressure breathing (−5 and −10 cm H_2O) produced an increase in breathing frequency. The changes were brought about by connecting the tracheal cannula to a large drum maintained at the required pressure. During stretch receptor block the inflation reflex was abolished and the deflation reflex persisted, confirming our earlier observations (Davies *et al.* 1978).

In a typical experiment the Breuer–Hering inflation reflex to +15 cm H_2O positive pressure lung inflation was measured as the ratio of the first expiratory time during lung inflation to control expiratory time (inhibitory ratio: i.r.). SO_2 was administered until the reflex was abolished, as indicated by an i.r. $\leqslant$ 1·0. The rabbit was paralysed and ventilated at the same frequency and tidal volume as its spontaneous breathing. This maintained normal end-tidal CO_2. Tidal volume was rapidly changed (within 2 or 3 pump cycles) and the new relationship between lung volume and phrenic activity recorded for about 60 sec. Tidal volume was returned to its original value and the Breuer–Hering inflation reflex again tested by connecting the expiratory port of the pump to a positive pressure of +15 cm H_2O. This procedure could be repeated using different test tidal volumes. It was never necessary to increase tidal volume by more than 30 % eupnoeic value to synchronize phrenic discharge with ventilation, usually with lung inflation. Stretch receptor block was allowed to wear off (30 min), as indicated by the return of the Breuer–Hering reflex, and the relationship between lung volume and phrenic activity again recorded. Block could be re-established by administering SO_2 via the pump. The animal was finally vagotomized.

In some experiments the rabbits were paralysed before giving the air-SO_2 mixture, which was administered through the ventilation pump. In these experiments the control condition, during paralysis but without stretch receptor block, could be recorded with the Breuer–Hering reflex at full strength.

Analysis of the phase relationship between the pump cycle and the phrenic bursts. We have considered only the onset of the phrenic burst in relation to the pump cycle. The two phases (inflation and deflation) of the pump cycle were divided in ten equal intervals, and the interval in which the phrenic burst started was identified (for example between 10 and 20 % of inflation). In each rabbit we analysed ten consecutive phrenic discharges in control conditions (i.e. with the rabbit paralysed, artificially ventilated and no blockade of stretch receptors), a further ten consecutive phrenic bursts during SO_2 block with the same pattern of ventilation, and ten bursts with increased tidal volume.

RESULTS

In all eight rabbits before blocking stretch receptors with SO_2 or when these receptors had recovered (as judged by the Breuer–Hering inflation reflex) the onset of phrenic discharge occurred within the first 70% of the lung deflation phase (Fig. 1, top record and Fig. 2).

When lung stretch receptors had been blocked by SO_2, as indicated by the disappearance of the Breuer–Hering inflation reflex, this 'out-of-phase' timing of

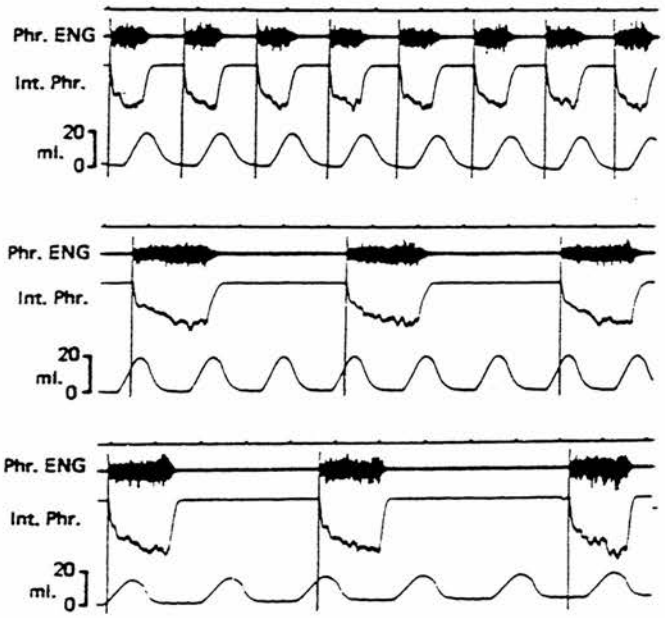

Fig. 1. Anaesthetized rabbit, paralysed and artificially ventilated. In each record from top: time in 1 sec intervals, phrenic electroneurogram, integrated phrenic. ENG and tidal volume (inflation upwards). *Top record:* control phrenic bursts starting in pump deflation. *Middle record:* after SO_2 administration with Breuer–Hering inflation apnoea totally inhibited and tidal volume and frequency as in control: phrenic burst now starting during pump inflation. *Bottom record:* as middle record, but tidal volume and pump frequency decreased: phrenic burst with no phase relationship to pump cycle.

phrenic discharge was invariably disrupted. In five of the eight rabbits there was initially no link between the phase of the ventilating pump and the onset of phrenic discharge. In this 'free running' situation mean phrenic discharge and pump frequencies were different (as in Fig. 1, bottom record). By increasing tidal volume the 'free running' condition could be converted to one in which a burst of phrenic activity was initiated by either of the pump's phases, more frequently by inflation. It was never necessary to increase tidal volume by more than 30% of the spontaneous eupnoeic value to abolish the 'free running' condition.

Conversely in the remaining three animals, when the SO_2 block had been established and control pattern of ventilation was maintained, the phrenic bursts

were immediately linked to either pump inflation (rabbits 4 and 5) or deflation (rabbit 8), and these timings were changed into a 'free running' pattern by decreasing the tidal volume (Fig. 1, middle and bottom records).

The times of onset of the phrenic discharges within the inflation and deflation phases of the ventilator, before and during lung stretch receptor block, with tidal

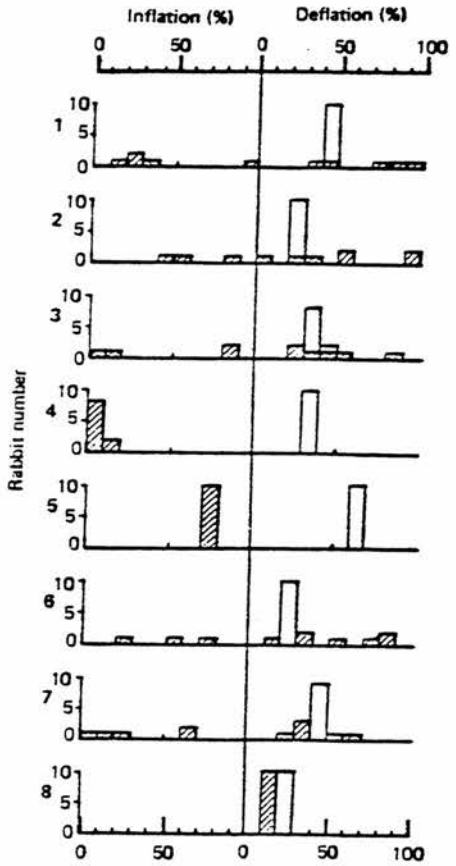

Fig. 2. The time of onset of the phrenic bursts in relation to the inflation and deflation phases of the ventilator. Each of the two phases is divided into equal intervals and the onsets of the phrenic bursts are identified in them. This analysis is applied to each of the eight experiments in the controls (open columns) and in total stretch receptor block (hatched columns). Tidal volume is the same, eupnoeic value in the control and blocked state. Ten successive phrenic discharges are included for each of the two conditions in each of the eight rabbits.

volumes at eupnoeic values are shown in Fig. 2. This illustrates the relegation of the onset of the phrenic burst to the deflation phase when stretch receptors are intact, and its occurrence either at random throughout the pump's cycle or linked to one of the two phases when they are blocked. When onset of phrenic activity was linked to one of the phases of ventilation it always occurred at the same time in that phase (Fig. 1 top record and Fig. 2, all rabbits, for deflation linking. Fig. 1, middle record, and Fig. 2 rabbit 5 for inflation linking).

Thus in the eight rabbits used phrenic activity was initiated in inflation in no rabbits when stretch receptors were intact; two rabbits when stretch receptors were blocked and V_T was at eupnoeic level; seven rabbits when stretch receptors were blocked and V_T was 30 % greater than eupnoeic level. Which is significantly different from the intact receptor state at the $P < 0{\cdot}01$ level. Phrenic activity was not restricted to the inflation phase of ventilation when it was initiated there.

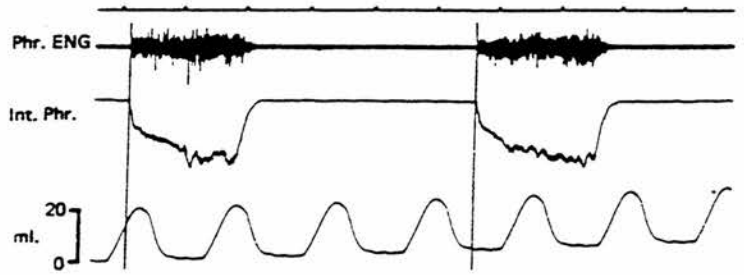

Fig. 3. Anaesthetized rabbit, paralysed and artificially ventilated. Both vagus nerves cut in the neck. Traces as in Fig. 1. There is no phase relationship between phrenic discharge and pump cycle.

After bilateral cervical vagotomy phrenic discharge was 'free running' with no set phase relationship to lung inflations of any volume or frequency. The frequency of phrenic discharge was less than during 'free running' before vagotomy (Fig. 3).

DISCUSSION

It is generally accepted that lung stretch receptor activity inhibits inspiration (see Bradley, 1977, for review). This negative feed-back mechanism results in phrenic discharge starting during the phase of lung deflation in artificially ventilated animals (Tang, Maire & Amassian, 1957). Block of lung stretch receptors by SO_2 modifies this temporal relationship. Single fibre recordings (Davies, 1976; Davies *et al.* 1978) show SO_2 can selectively abolish pulmonary stretch receptor activity in rabbits. There is a concomitant loss of the Breuer–Hering reflex at all levels of lung inflation. The onset of phrenic activity during inflation in the present experiments further suggests the absence of any inspiratory inhibiting influence that increases with lung inflation.

During SO_2 block, with passive ventilation maintained at its previous control level, the most frequent pattern was one of total independence between pump and phrenic cycles. This 'free running' indicates the absence of any sort of feed-back: the inspiratory output is driven neither by any of the remaining vagal influences nor by extravagal inputs. In a minority of the experiments the SO_2 inhalation, with abolition of the Breuer–Hering reflex, could not unlink the phrenic bursts from the pump cycle: it was usually in the inflation phase that the inspiratory discharge was initiated, but sometimes it occurred in the earlier part of deflation. Similar 'triggered' patterns could be caused by a moderate increase in tidal volume during 'free running' and, conversely, the triggered pattern could be reversed into 'free running' by a decrease in tidal volume.

On the other hand the 'free running' after bilateral vagotomy could not be altered by changes in the ventilatory pattern. These results suggest the presence of a vagal inspiratory initiation influence, exposed by the block of pulmonary stretch receptors, sometimes active with levels of ventilation similar to quiet breathing, and consistently induced by a slight increase in ventilation. It is impossible to say whether the increase in tidal volume or in airflow was responsible for the triggering since both were increased.

This coincidence of phrenic discharge with either inflation or deflation or both during stretch receptor block was probably due to lung irritant receptors which are still active at that time, as shown by fibre recording of action potentials (Davies *et al.* 1978). The influence of this receptor activity on pattern of breathing was demonstrated by cutting the vagi during stretch receptor block: this caused an increase in expiratory duration, a change consistent with the view that the non-blocked irritant receptors shortened expiration or triggered the next inspiration. These receptors were further demonstrated to be active by their accelerating effect on breathing during lung inflation or deflation (Sellick & Widdicombe, 1970). It is unlikely that the sensory endings of nonmyelinated fibres can account for the initiation of inspiration in our experiments. Guz & Trenchard (1971) have demonstrated that J-receptors have no tonic reflex effect on breathing in rabbits with healthy lungs. Coleridge & Coleridge (1977) point out these receptors 'may be a heterogeneous collection of endings' and therefore be activated by a variety of stimuli. However the natural stimulus suggested by Paintal (1973) for J-receptors is 'pulmonary congestion'; the degree of inflation needed to stimulate the receptors for cats (Armstrong & Luck, 1974), rabbits (Sellick & Widdicombe, 1970) and dogs (Coleridge *et al.* 1965), and the 'sparse and irregular' activity of both bronchial and pulmonary C-fibres reported by Coleridge & Coleridge (1977) for dogs militates against, but does not exclude, the possibility of their initiating inspiration under conditions of near eupnoeic tidal volume.

Brief intense bursts of irritant receptor activity provoke an augmented inspiration (Davies & Roumy, 1978). This reponse appears to be 'all-or-none', the augmented inspiration being present or absent. However another role of irritant receptors is to shorten expiration (Davies, 1978). While we suggest that irritant receptors can initiate inspiration we have no evidence for their effects, if any, on its duration. In a previous study (Davies *et al.* 1978) we could detect no change in the rate of increase of integrated phrenic activity when pulmonary stretch receptors were blocked by SO_2 or when manoeuvres which stimulated irritant receptors were carried out. Lung irritant receptor activity seemed to terminate or extend phrenic activity which had a rate of increase already 'predetermined', a conclusion reached by Winning & Widdicombe (1976) using cats and supported by our previous observations on rabbits (Davies, 1976).

What contribution might irritant receptors make to the onset of inspiration under physiological conditions? Irritant receptor activity exists at functional residual capacity and the tonic activity of stretch receptors at this level of lung inflation is also low. Thus the onset of inspiration may not only depend on a waning central inhibition as suggested by Clark & Euler (1972) but may also be influenced by an inspiratory initiating drive from lung irritant receptors. This activating mechanism

could be at work in patterns of breathing without an expiratory pause where an inspiration starts immediately after end-expiratory level has been reached. During the phase of expiratory flow stretch receptor activity is small and irritant receptors are usually active, conditions favourable for inspiratory initiation.

Therefore under the conditions of our experiments irritant receptor activity together with activity in un-myelinated vagal fibres could play a part in the initiation of inspiration. This role is compatible with the effects of irritant receptors in shortening expiration and provoking augmented breaths if the initiation of inspiration is considered as a termination of expiration.

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Lung

Patterns of Respiratory Response of Rabbits to Histamine

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Abstract. We analysed the respiratory response of anaesthetised rabbits to aerosols or injections of histamine. Shortening of expiratory duration (t_E) could preceed shortening of inspiratory duration (t_I) by a few breaths (continuous acceleration) or t_I could remain constant until an augmented breath preceded a large reduction in t_I (discontinuous acceleration). The responses were not appreciably modified by a block of pulmonary stretch receptors and are therefore attributable to increased activity of lung irritant and, to a lesser extent, C-fibre receptors. The results are discussed in relation to the mechanisms controlling t_I and t_E. We conclude that such mechanisms are relatively independent under certain circumstances and that irritant receptors may have an indirect role in shortening t_I.

Key words: Receptors, sensory – Respiratory system – Lung – Histamine

Introduction

Increased breathing frequency (f) can be due to shortening of inspiration (t_I), shortening of expiration (t_E) or a combination of both. Recent studies have shown that histamine causes tachypnoea in rabbits, shortening t_E and t_I, by vagally mediated lung reflexes. [3, 5, 7, 12].

Histamine given by aerosol or intravenously causes an increase in activity of all three systems of lung receptors, stretch receptors [8, 9, 15], irritant receptors [9, 11, 14] and bronchial C-fibre receptors [2].

The contribution of lung receptors with vagal C-fibres to the tachypnoea due to intravenous histamine is rather small [6, 16], since differential block of myelinated vagal fibres blocks the effect. In a previous study, intravenous histamine had essen-

tially the same overall effect on t_E whether or not pulmonary stretch receptors were blocked by SO_2. On the other hand "histamine produced a small statistically insignificant decrease in t_I" when stretch receptors were intact which was abolished by their block and, "in many cases t_I slightly increased" [5]. In the present experiments different conditions produced different results.

Recent experiments [3, 4] show that lung irritant receptors in rabbits are mainly involved in the control of t_E and their only direct effect on inspiration is to prolong it into an augmented breath. What then can be the mechanism which shortens t_I in experiments which include a total block of pulmonary stretch receptors? Using a well validated method of blocking pulmonary stretch receptors we have analysed the time course of changes in t_I and t_E and determined the influence of spontaneous or induced augmented breaths on histamine provoked changes in pattern of breathing.

Methods

Experiments were carried out on nine New Zealand white rabbits (weight 2.5–4.0 kg) anaesthetized by pentobarbitone sodium (initial does 40 mg/kg intravenously). Surgical preparation consisted of insertion of a polyethylene cannula into the trachea, catheters into a femoral artery and vein and an intrapleural catheter. The uppermost root of the right phrenic nerve was exposed and cut distally.

Air flow was recorded from a Fleisch pneumotachograph head connected to the tracheal cannula. Flow was integrated electronically to obtain tidal volume. Total lung resistance and compliance were determined by the method of Mead and Whittenberger [10].

Electrical activity was recorded from multifibre preparations of the cut root of the right phrenic nerve and used for the measurement of inspiratory and expiratory durations. Using the system described by Winning and Widdicombe [17] t_I was taken as the interval between initial increase and the start of the rapid decrease of integrated phrenic activity. t_E was the remainder of the respiratory cycle.

Histamine was either given as an aerosol of 2% solution of the di-hydrochloride (mean particle dia 8 μm, exposure time 30s) or injected into the femoral vein as the acid phosphate (50–150 μg/kg).

Aerosol was generated by a model 65 de Vilbiss ultrasonic nebulizer. A low pressure stream of air carried the aerosol, via a wide bore tube, to a T-piece attached to the pneumotachograph. Movement of air and the short arms of the T-piece prevented an increase in dead space. Aerosols and injections of physiological saline were used as controls. Animals were allowed to recover for 30 min between tests to avoid tachyphylaxis.

Variables (blood pressure, air flow, tidal volume, phrenic electroneurogramm and intrapleural pressure) were recorded by a u.v. recorder and on magnetic tape.

Pulmonary stretch receptors were selectively blocked by making the anaesthetized rabbits breath 200 ppm SO_2 for at least 10 min [5]. Block was judged complete when the Hering-Breuer inflation reflex to lung inflation by a pressure of 10 cm H_2O was abolished. The block was tested after each administration of histamine. Results are not included if the block was not complete. Block usually lasted 20–30 min.

In some experiments brief inflations or deflations of the lungs were used to provoke augmented breaths immediately before administration of histamine. Inflation or deflation was produced by an electromagnetic valve which briefly (100 ms) connected the tracheal cannula to a large drum maintained at the required pressure (± 20 cm H_2O) [4].

Changes in end-expiratory level of the tidal volume (V_T) trace (which was adjusted to minimize integrator drift) were taken as changes in FRC. Results are the means of five measurements before and at the peak of response to administration of histamine. Statistical analysis is based on paired values (before and after intervention) by two tailed t-test, n values refer to numbers of tests.

Results

Aerosols of physiological saline administered under the same conditions as histamine produced a small insignificant increase in t_E. Inhalation of histamine aerosol before ($n=32$) or during SO_2 block ($n=9$) always caused an increase in f ($+50\%$ to $+120\%$), a reduction in V_T (-20% to -50%) and an increase in FRC (5–10 ml). Lung compliance decreased from a mean of 4.9–2.5 ml cm H_2O^{-1} and total lung resistance increased from 30 to 50 cm H_2O. l^{-1} s. The respiratory response to histamine was prevented by vagotomy ($n=3$ rabbits).

Augmented breaths occurred spontaneously. They consisted of a longer and deeper inspiration followed by shortening of t_I and t_E for a few breaths. Augmented breaths were quite distinctive. In a previous study [4] we found t_I of augmented breaths to be $1.85 \pm 0.22\times$ control values and so easily identifed.

As shown previously [5] abolition of stretch receptor activity increased t_I and V_T and decreased f; t_E changes were variable. The reactions to histamine in terms of maximal change showed no significant difference before and after SO_2 (Table 1).

Table 1. Incidence of continuous and discontinuous acceleration in nine rabbits before and during block of pulmonary stretch receptors. t_I, t_E and V_T (mean±S.D.) measured at rest and at maximum frequency change. There was no significant difference in these variables at maximum frequency whether acceleration was continuous or discontinuous

	Control		SO_2 Block	
	Rest breathing	Histamine aerosol	Rest breathing	Histamine aerosol
Continuous acceleration	10		5	
Discontinuous acceleration	22		4	
t_I (s)	0.46 ± 0.05	0.33 ± 0.04	0.80 ± 0.16	0.51 ± 0.06
t_E (s)	0.83 ± 0.32	0.35 ± 0.17	0.99 ± 0.30	0.38 ± 0.08
V_T (ml)	23.1 ± 3.63	15.7 ± 4.56	30.2 ± 2.78	19.4 ± 4.85

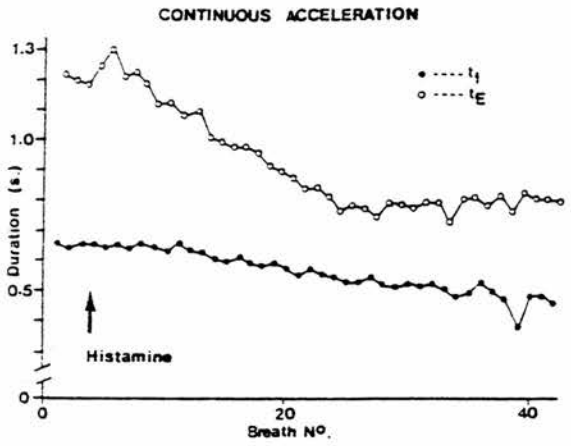

Fig. 1. Breath by breath plot of inspiratory duration (t_I) and expiratory duration in a rabbit inhaling histamine aerosol

The first change in breathing was always a shortening of t_E. After a delay os several breaths (mean = 12 for aerosol and 3.2 for injection) t_I started to change. Some times the change in t_I was smooth and gradual or there was no change in t_I until abruptly initiated by an augmented breath. In such a pattern the mean t_I of t_E of the five breaths immediately before the augmented breath was not significantly different from the preceeding five control breaths. The five breaths immediately following the augmented breath were significantly different from control and this difference persisted while histamine was applied. For convenience these patterns will be called "continuous" and "discontinuous acceleration". Acceleration always involved a shortening of t_I and t_E. The presence of the easily identifed augmented breath characterised the discontinuous type. The remainder were continuous. Both types occurred whether stretch receptors were blocked or intact. With receptors intact continuous acceleration occured 10 times (31%) and discontinuous acceleration 22 times (69%). During receptor block the occurrences were five and four times respectively. The differences was not statistically significant (χ^2-test).

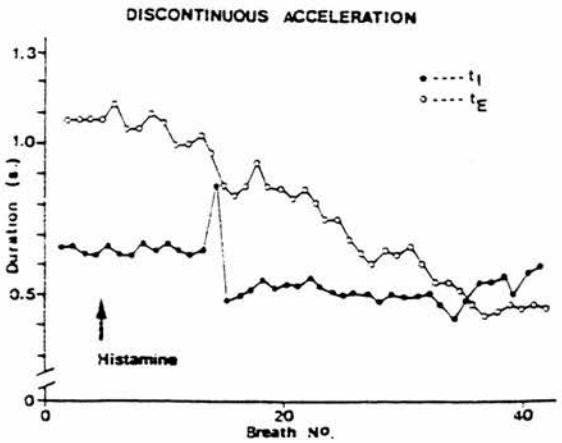

Fig. 2. See Fig. 1

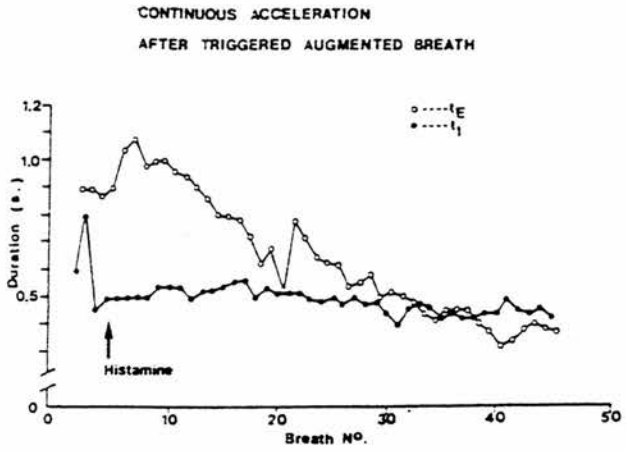

Fig. 3. See Figs. 1 and 2. At breath 1 an augmented breath was triggered by a brief inflation pulse and immediately followed by the histamine aerosol

An example of continuous acceleration is shown in Fig. 1. The small increase in t_E at the begining of histamine aerosol administration, also seen in Figs. 2 and 3, may be due to connection of the tracheal cannula to the aerosol apparatus since it was also observed with saline controls. t_I started to shorten later than did t_E and the decrease was gradual. Figure 2 shows an example of discontinuous acceleration in the same rabbit. t_I remained unchanged until an augmented breath occurred, after which t_I was immediately and substantially shortened.

An augmented breath is followed by a refractory period in which it is impossible to provoke further augmented breaths [13]. Davies and Roumy [4] have demonstrated that this refractoriness is restricted to t_I. In 10 instances we provoked an augmented breath by brief inflation or deflation of the lungs immediately before histamine administration. The effects produced by the intervention were similar to histamine – provoked discontinuous acceleration (Fig. 3).

Injected histamine was used in five rabbits before SO_2 block (three discontinuous, two continuous accelerations produced) and in four rabbits during SO_2 block (two continuous, two discontinuous). The only difference from histamine aerosol was the speed of effect. t_E was shortened to its minimum value in three or four breaths.

Discussion

As part of a previous series of experiments [5] we used intravenous histamine to accelerate breathing of anaesthetized rabbits. Acceleration was mainly due to a reduction of t_E which could be produced even when pulmonary stretch receptors (p.s.r.) were selectively blocked by SO_2. t_I was not significantly shortened before block and insignificantly increased during block. The difference between these results and those of others [12] who found t_I substantially shortened by histamine aerosol may be due to the conditions from which acceleration was inititated. In our experiments t_I of lightly anaesthetized rabbits was 0.48 s before and 0.6 s during p.s.r. block. Miserocchi et al. [12] reported t_I to be between 0.7 and 0.8 s under con-

trol conditions with p.s.r. intact (Fig. 2, [12]) but did not carry out a selective block of these receptors.

In our present experiments we used histamine aerosol to accelerate breathing of anaesthetized rabbits from a lower initial frequency and found that while acceleration is still mainly due to a reduction in t_E, t_I can be shortened before and during p.s.r. block. Furthermore, histamine accelerated breathing in rabbits can be of one of two types, both of which can occur in the absence of p.s.r. activity. It is generally accepted that p.s.r. activity shortens t_I [1]. Table 1 shows that t_I can be shortened whether p.s.r.'s are blocked or intact. We have previously demonstrated [3, 4] that irritant receptor activity shortens t_E and its only effect on t_I is to provoke an augmented breath i.e. a large increase in t_I. This is an all or none effect: levels of irritant receptor stimulation during inspiration which do not provoke an augmented breath do not alter t_I. If irritant receptor activity extends t_I, as in an augmented breath, what shortens t_I during histamine administration? The contribution of receptors with vagal C-fibres is rather small [6, 16] since the effect is abolished by differential block of myelinated vagal fibres. We suggest that a possible linking of the central mechanisms which determine t_I to those which control the already shortened t_E may bring about the shortening of t_I. It is clear from the way changes in t_E preceed those of t_I in continuous acceleration, and from the existance of discontinuous acceleration, that t_I and t_E are independently controlled over at least part of their range. That they can be linked is suggested by the major part of continuous acceleration. This linking may be a direct of a shortened t_E on t_I, or the response of the independent neural systems controlling these variables to a common input. If the latter is the case sensitivity of the mechanism controlling t_I must change dramatically after an augmented breath where t_I changes from control to near minimal value in a matter of one or two breaths. What brings about this change or linking? It cannot be due to stretch receptors as it occurs whether they are active or blocked. It may be due to the activity or irritant receptors. However, the only direct influence of irritant receptor activity on t_I we have been able to demonstrate is the production of an augmented breath [4] which consists of a lengthened t_I. We therefore suggest that irritant receptor activity in discontinuous acceleration does not act directly on the neural mechanisms governing t_I when t_I is being shortened, rather it promotes a link between these mechanisms and those governing the already shortened t_E.

Further implication of irritant receptors in the shortening of t_I is seen in Fig. 3. Here an augmented breath was provoked by stimulating irritant receptors with a brief pulse of lung inflation [4]. When this stimulation was sustained by histamine aerosol the pattern of breathing was closely similar to discontinuous acceleration provoked by histamine alone.

Other workers have generally not commented on the types of tachypnoea produced by histamine in their experiments, probably because the sampling techniques used concentrated attention on the maximum effect produced. The closest type of analysis to that used in the present study was that of Winning and Widdicombe [17] who used intravenous histamine in cats. Although they did not comment on the existence of discontinuous acceleration, the bottom panel in Fig. 5 of their paper shows a discontinuity between 2.2 and 1.2 s.

Our present experiments show that in rabbits, with or without pulmonary stretch receptor activity, t_E can change independently of t_I over part of its range, and that

breathing in the presence or absence of stretch receptor activity can accelerate in one of two distinctly different ways. We suggest that in such acceleration of breathing rapidly adapting "irritant" receptors act indirectly to shorten t_I in the absence of pulmonary stretch receptor activity.

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THE EFFECT OF TRANSIENT STIMULATION OF LUNG IRRITANT RECEPTORS ON THE PATTERN OF BREATHING IN RABBITS

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SUMMARY

1. 100 ms pulses of inflation and deflation were applied to the lungs of anaesthetized rabbits before and during inactivation of pulmonary stretch receptors.

2. Pulses of either sign given in inspiration often produced augmented breaths, whether or not stretch receptors were inactivated. Inflation pulses were more effective than deflation pulses.

3. After an augmented breath it was impossible to produce another for at least 1 min (refractoriness).

4. Pulses of deflation always shortened expiration. Pulses of inflation early in expiration shortened expiration. Later in expiration they lengthened expiration when stretch receptors were active and shortened expiration when stretch receptors were blocked. No refractoriness was observed for the effects on expiratory time.

5. Pulses in paralysed animals caused a burst of phrenic activity of fixed duration, usually with brief latency. There was no refractoriness.

6. We suggest that the duration of inspiration is governed by the activity of pulmonary stretch receptors, except during an augmented breath, and that the duration of expiration is governed by a balance of stretch and irritant receptor activity.

INTRODUCTION

Augmented breaths, whether spontaneous or triggered by inflation of the lungs, have been ascribed at least in part to the excitation of lung receptors (Reynolds, 1962), probably 'irritant' receptors (Sellick & Widdicombe, 1970; Glogowska, Richardson, Widdicombe & Winning, 1972). However, in cats histamine, which stimulates irritant receptors when given either intravenously or as an aerosol, accelerates breathing (Karczewski & Widdicombe, 1969) with a decrease in inspiratory (T_I) and expiratory durations (T_E) (Widdicombe & Winning, 1976), which suggests that irritant receptors shorten both T_I and T_E. Knox (1973) reported a decreased T_E due to pulses of deflation

given during expiration; he ascribed this effect to the excitation of irritant receptors, a conclusion in agreement with the decrease of T_E produced by histamine. However, part of this response to deflation could be due to decreased stretch receptor activity (Knox, 1973; Bartoli, Bystryzcka, Guz, Jain, Noble & Trenchard, 1973).

Since the majority of lung stretch receptors and all their reflex effects can be blocked by inhalation of SO_2 (Callanan, Dixon & Widdicombe, 1975; Davies, 1976), we re-investigated the responses to irritant receptor stimulation by pressure pulses in intact and SO_2-treated rabbits.

METHODS

We used seven New Zealand White rabbits weighing between 2·0 and 4·0 kg. Anaesthesia was induced and maintained with sodium pentobarbitone (40 mg/kg, Nembutal, Abbot). Three rabbits were in addition paralysed with 60 mg of gallamine triethiodide, and artificially ventilated. A polyethylene cannula was tied into the trachea, and polyethylene catheters were tied into a femoral vein and artery. Blood pressure was monitored from the arterial catheter by a strain-gauge transducer (Consolidated Electrodynamics).

Tidal volume (V_T) was measured by electronically integrating air flow measured by a Fleisch pneumotachograph head connected to the tracheal tube.

Phrenic activity was recorded from multifibre strands of the upper cut root of the right phrenic nerve, placed in a trough of liquid paraffin; two platinum electrodes and a Tektronix 122 amplifier were used. The raw signal was integrated by a non-leaky amplifier (Davies & Wise, 1978). T_I and T_E were measured from the initial increases and the starts of the rapid decreases of phrenic activity. Activity from nerve fibres with discharge patterns which identified them as lung irritant or stretch receptors was recorded from 'single fibre' preparations of the right vagus nerve in three rabbits. The left vagus was intact. Instantaneous frequency of discharge of receptors was calculated by measuring the time interval between successive spikes.

Pulses of inflation and deflation of the lungs were obtained by briefly connecting the tracheal cannula to a large (20 l) drum, which was maintained at positive or negative pressure, by means of solenoid-operated valves which opened for the required pulse duration. The valves were triggered manually and the distribution of pulses throughout the respiratory cycle was randomized and explored all parts of the cycle. Pulses of 100 ms and $\pm 2{\cdot}0$ kPa pressure were found to be effective and produced inflations of $+20{\cdot}85 \pm 5$ ml (mean $\pm$ S.E.M.) above end-expiratory volume ($n = 34$, two rabbits) and deflations of $-6{\cdot}19 \pm 0{\cdot}4$ ml ($n = 34$, two rabbits) when tidal volume was $18{\cdot}87 \pm 4{\cdot}9$ ml ($n = 68$, two rabbits). End-tidal P_{CO_2} was monitored by a Beckman infra-red analyser and kept near the control value ($38{\cdot}2 \pm 1{\cdot}8$ torr) in intact and paralysed animals.

In some experiments stretch receptor activity was blocked by causing the animal to breath 200 parts per million SO_2 in air for 10 min (Callanan *et al.* 1975). Complete abolition of the Breuer–Hering inflation reflex (the prolongation of T_E caused by inflation of the lungs with a constant pressure of 1·5 kPa) was taken as evidence of stretch block. The responses to pulses of inflation and deflation were compared before and during stretch receptor block. The variables in each experiment were recorded on an ultraviolet recorder (Southern Electronics 6008) and on a seven-channel magnetic tape recorder (Ampex). Results are given as mean $\pm$ S.E. of the mean unless otherwise stated. Student's t test was used to evaluate the significance of differences. N-values refer to numbers of tests.

RESULTS

Spontaneously breathing rabbits

Pressure pulses during inspiration

Pulses of inflation or deflation given during inspiration often caused an augmented breath with an increase in V_T and T_I and a decrease in T_E (Fig. 1). 36% of inflation pulses and 7% of deflation pulses caused augmented breaths. In fifty-seven measured

augmented breaths in seven rabbits, T_I was $0{\cdot}73 \pm 0{\cdot}09$ s compared with immediately previous controls of $0{\cdot}41 \pm 0{\cdot}115$ s ($P < 0{\cdot}001$), a ratio of $1{\cdot}85 \pm 0{\cdot}22$. The response was all-or-none, no gradation occurring when the pulse characteristics were varied. In most cases the V_T and phrenic integral slopes were clearly biphasic.

The T_E immediately following augmented breaths was decreased (from $0{\cdot}72 \pm 0{\cdot}04$ s to $0{\cdot}52 \pm 0{\cdot}1$ s), the ratio to the preceding control being $0{\cdot}72 \pm 0{\cdot}17$ ($P < 0{\cdot}001$). Both

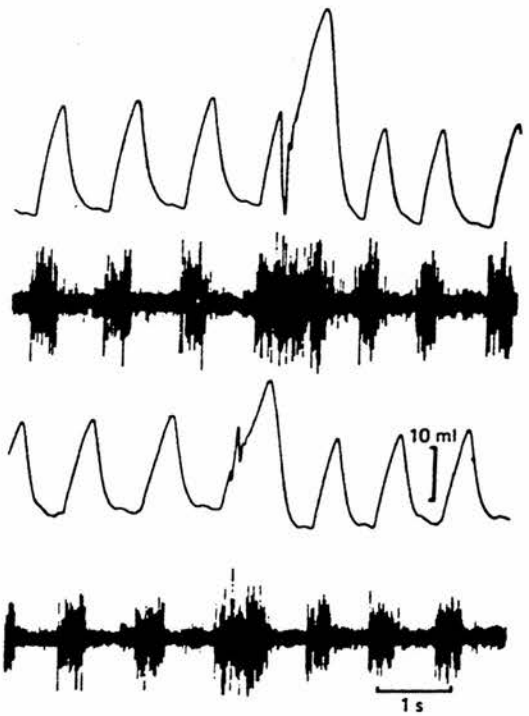

Fig. 1. Augmented inspiratory responses triggered by pulses of deflation (top trace) and inflation (lower trace). In each case tidal volume is shown above phrenic discharge.

T_I and T_E of the next subsequent breath were reduced (T_I ratio, $0{\cdot}87 \pm 0{\cdot}1$; T_E ratio, $0{\cdot}85 \pm 0{\cdot}1$), and returned to control values over two or three breaths.

Once an augmented breath had been elicited it was impossible to trigger another within 1 min; but after 2 min augmented breaths could usually be obtained. An augmented breath was more readily triggered by a pulse in the first half than by one in the second half of inspiration (Table 1). Spontaneous augmented breaths were never observed less than 1 min after a triggered augmented response. This refractoriness is thought to explain the absence of augmented breaths after many of the pulses in the whole series of experiments.

When a pulse produced no augmented breath, there was no significant change in T_I; however measurements in four rabbits showed that the following T_E was reduced (inflation pulses; T_E from $0{\cdot}66 \pm 0{\cdot}06$ s to $0{\cdot}54 \pm 0{\cdot}12$ s, $n = 51$, $P < 0{\cdot}01$; deflation

pulses: T_E from 0·66±0·12 to 0·58±0·18 s, $n = 40$, $P < 0·01$). The following T_I was not statistically significantly different from the control.

Pulmonary stretch receptors were blocked by administering SO_2 (200 p.p.m. in air) for 10 min (Callanan *et al.* 1975; Davies, 1976). The block was assessed by the complete disappearance of the Breuer–Hering reflex. In receptor-blocked rabbits,

TABLE 1. Numbers of triggered augmented breaths (t.a.b.s) produced by pulses to the lungs of four rabbits. Equal numbers of inflation and deflation pulses were applied, but more were given in the first half of inspiration. * $P < 0·01$ for the difference in ratio between the first and second half of inspiration (χ^2 test)

	Stretch receptors intact	
	First half	Second half
Pulses	120	41
T.a.b.s	48	4
Ratio	0·4	0·1*
	Stretch receptors blocked	
	First half	Second half
Pulses	110	21
T.a.b.s	18	1
Ratio	0·16	0·05

pressure pulses still caused augmented breaths (control T_I, 0·63±0·10 s; augmented T_I, 1·02±0·18 s; ratio 1·67±0·17; $n = 19$, $P < 0·01$); however the responses were more difficult to elicit, only 15% of inflation pulses being effective compared with 36% without block. Refractoriness after augmented breaths was still present. Pulses in the first half of inspiration were more effective in causing augmented breaths, although the difference was not statistically significant (Table 1). During stretch receptor block T_E after the augmented breath was decreased (ratio 0·66±0·17; $n = 19$, $P < 0·01$).

During stretch receptor block, when no augmented breath was obtained, pressure pulses did not significantly change T_I; however they decreased T_E (ratio 0·77±0·16, $n = 47$, $P < 0·01$).

No responses were obtained in rabbits without stretch receptor block when pulse pressure was set to atmospheric (twelve tests in six rabbits), or after cervical vagotomy (six rabbits).

Pressure pulses during expiration

Pulses of deflation decreased T_E. Pulses early in expiration usually reduced T_E by 80–90%. To quantify this effect we calculated $(T_{E,p} - T_p)/(T_{E,c} - T_p)$ where $T_{E,p}$ is the duration of the expiration containing the pulse, T_p is the delay between the end of the previous phrenic discharge and the beginning of the pulse and $T_{E,c}$ is the control expiratory duration (Fig. 2); thus the term is the ratio of the duration of expiration after the pulse to the potential duration of expiration after the pulse if the pulse had had no effect, calculated from the previous expiration. If the term is greater than unity then T_E has been increased, if less than unity, then T_E has been decreased. The term is plotted against $T_p/T_{E,c}$, the position of the pulse in expiration. If the pulses terminated expiration with a constant latency, i.e. with a constant $(T_{Ep} - T_p)$, the

plot would describe a segment of a hyperbola (Fig. 2, interrupted lines), the effect of this termination being most conspicuous with pulses at the beginning of expiration. Assuming a latency of $(T_{E,p}-T_p) = 0{\cdot}1\ T_{E,c}$, (a value frequently obtained experimentally) we constructed the interrupted lines of Fig. 2. Shortening of T_E produced by pulses of deflation gave values close to the curve indicating that the pulses

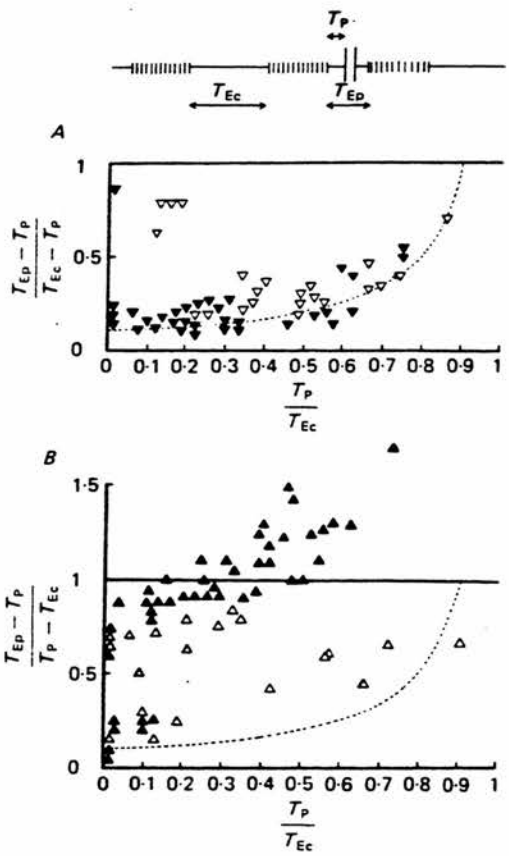

Fig. 2. Effect on T_E pulses of deflation (*A*) and inflation (*B*) given during expiration. The interrupted line was calculated assuming that the shortening of T_E measured as T_E-T_p was constant and equal to 0·1 $T_{E,c}$. In both cases the filled triangles were obtained with intact animals and the open triangles in animals with lung stretch receptors blocked by SO_2.

triggered the next inspiration with near-constant latency (Fig. 2*A*) (other latency isobars (omitted for clarity) may be drawn and have similar shape). During block of pulmonary stretch receptors by SO_2 the responses to pulses of deflation were not very different.

In four deflations during stretch receptor block and one deflation without block the shortening of T_E was small (Fig. 2*A*). The pressure pulses were in a phase of rapidly decreasing lung volume and may have produced little receptor stimulation.

Pulses of inflation at the beginning of expiration usually shortened T_E (Fig. 2*B*), sometimes with a latency as short as 0·1 s. Pulses later in expiration had little effect on T_E, and even later the response reversed to an increase in T_E. After block of pulmonary stretch receptors with SO_2 only shortening of T_E was obtained with pulses of inflation, the values sometimes being near to the curve of constant latency (Fig. 2*B*).

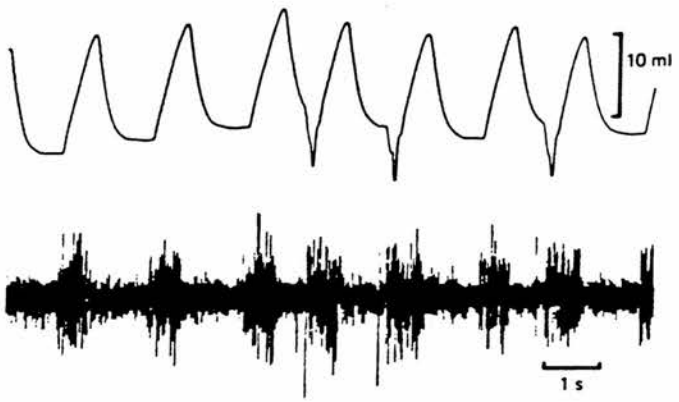

Fig. 3. Three pulses of deflation given in rapid succession in expiration. There is each time a shortening of expiration. Top trace = tidal volume. Bottom trace = phrenic discharge. The horizontal bars indicate pulse timing.

Pulses repeated in consecutive expirations, with stretch receptors blocked or intact, repeatedly produced a shortening of T_E (Fig. 3), demonstrating the absence of refractoriness.

When T_E was shortened by pressure pulses the following T_I was decreased except on rare occasions when a pulse of deflation late in expiration triggered an augmented breath. The size of the change in T_I was not closely correlated to the change in T_E.

Recordings from lung receptors

We recorded activity from twelve vagal fibres which showed patterns typical of pulmonary stretch or lung irritant receptors (Adrian, 1933; Mills, Sellick & Widdicombe, 1969, 1970). They all responded to changes in volume of the lungs and were unlikely to be in the extrathoracic trachea since the tracheal cannula was inserted as low as possible in the neck. Those designated 'stretch receptors' had low-volume thresholds, slowly adapting discharges to maintained lung inflations and decreases in any spontaneous activity with lung deflations. Those designated lung 'irritant receptors' responded to maintained lung inflations and deflations with rapidly adapting irregular discharges.

Seven stretch receptors briefly increased discharge during pulses of inflation (mean +47 %, $n = 12$ pulses) and decreased discharge during pulses of deflation (−45 %, $n = 12$) (Table 2). When the pulse triggered an augmented breath there was a large increase in firing frequency during the second part of the breath coincident with the increase in tidal volume. When the pulse did not trigger an augmented breath the total number of spikes in a respiratory cycle was little affected (about 5–10 % change).

Five irritant receptors responded to both pulses of inflation (+487 %) and deflation (+463 %) with intense burst of activity (Table 2, Fig. 4). Pulses of deflation produced the largest excitations when given at functional residual capacity (f.r.c.), during the expiratory pause. For example, a receptor studied in this respect discharged 17·5±2·8 impulses in the 0·1 s of the pulse of −2 kPa at f.r.c., compared

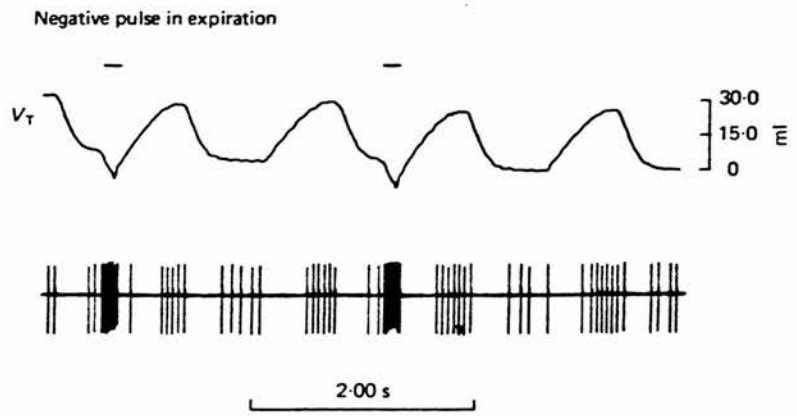

Fig. 4. Tidal volume and activity of a single lung irritant receptor when pulses of negative pressure were applied during expiration. The horizontal bars indicate pulse position.

TABLE 2. Number of impulses produced by stretch and irritant receptors during pulses of inflation and deflation compared with the number of impulses in the same time interval of the previous breath (control period)

Receptor type	Pressure pulse	No. of impulses in control period	No. of impulses in pulse	Change
Stretch: 12 tests 7 receptors	Positive	6·8±0·45	10·0±0·69	+3·2 (+47 %)
	Negative	8·2±0·69	4·5±0·33	−3·7 (−45 %)
Irritant: 11 tests, 5 receptors	Positive	1·67±1·0	9·8±2·0	+8·13 (+487 %)
	Negative	1·83±1·0	10·3±2·6	+8·47 (+463 %)

with 0·5±0·5 impulses in the corresponding control period (Table 3); at 70 % V_T the same receptor was not affected by equivalent pressure pulses. On the other hand, pulses of inflation at the peak of tidal volume were highly effective in producing activity. Thus five inflation pulses of 2 kPa given at 90 % tidal volume produced 9·2±0·71 impulses in the 0·1 s of the pulse, compared with a control value of 1·5±0·9 impulses.

During augmented breaths irritant receptor discharge was increased in amount and duration; for five augmented breaths the mean total number of impulses was 74·4±22·3 compared with a control of 14·6±8·0 (five receptors).

During the period after an augmented breath, corresponding to refractoriness, irritant receptors were still excited by pressure pulses, with no clear changes in sensitivity.

Spontaneous augmented breaths

In all spontaneously breathing rabbits, spontaneous augmented breaths had similar characteristics to the triggered responses. The ratio T_I to control was $1{\cdot}88 \pm 0{\cdot}21$ ($n = 24$), not significantly different from the ratio of triggered responses ($1{\cdot}85 \pm 0{\cdot}22$). In any particular animal, the tidal volumes of spontaneous and triggered augmented breaths were similar. Although the following T_E was usually decreased, it was occasionally longer, and the mean was not significantly different from control.

TABLE 3. Effect of positive and negative pulses on the activity of an irritant receptor at different lung volumes

Lung volume	Pressure pulse	Impulses during pulse
F.r.c.	None (control)	$2{\cdot}5 \pm 0{\cdot}9$
	Positive	$3{\cdot}6 \pm 1{\cdot}0$
90% V_T	None (control)	$1{\cdot}5 \pm 0{\cdot}9$
	Positive	$9{\cdot}2 \pm 0{\cdot}7$
F.r.c.	None (control)	$0{\cdot}5 \pm 0{\cdot}5$
	Negative	$17{\cdot}5 \pm 2{\cdot}8$
70% V_T	None (control)	$2{\cdot}9 \pm 0{\cdot}6$
	Negative	$2{\cdot}9 \pm 1{\cdot}3$

Five pulses of inflation and five of deflation were given at high and low lung volumes. The number of impulses during the pulse are compared with the number during the same time interval, at the same level of tidal volume in the previous (control) breath.

The spontaneous augmented breaths occurred, but were not usually recorded, at intervals of about 5 min. During block of pulmonary stretch receptors, frequency decreased to one or two augmented breaths per hour. They were never seen in bilaterally vagotomized rabbits.

In three rabbits we applied pressure pulses to the lungs during inspiration as soon as possible after spontaneous augmented breaths. In none of seventeen tests was an augmented breath produced. The refractoriness lasted 1–2 min.

Artificially ventilated rabbits

Three paralysed anaesthetized rabbits were ventilated with tidal volumes and frequencies as near as possible to their spontaneous patterns of breathing. Phrenic discharge locked to the pump frequency and occurred during deflation of the lungs.

Pulses of deflation given during the phrenic pause triggered a burst of phrenic activity lasting about 0·31 s, or $0{\cdot}6 \pm 0{\cdot}02$ ($n = 8$) times the control T_I, and starting 0·08–0·09 s from the beginning of the pulse (Fig. 5). The burst was all-or-none, not depending on pulse characteristics. Pulses of inflation during the phrenic pause produced a similar burst of activity but always at peak tidal volume (Fig. 5).

Pulses given during phrenic activity lengthened the discharge (Fig. 6), the effect being greatest with pulses towards the end of the discharge. This is consistent with the pulse causing a burst of phrenic activity of constant duration after a very short latency either within the spontaneous activity or else prolonging it.

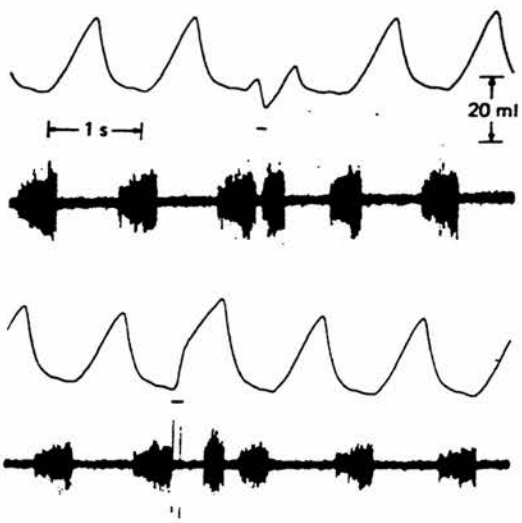

Fig. 5. Effect of pulses of deflation (above) and inflation (below) given during phrenic 'expiration' in an anaesthetized paralysed artificially ventilated rabbit. The artifact produced by the opening and closing of the magnetic valves is clearly seen on the phrenic record during the inflation pulse. In both cases the upper trace is tidal volume and the lower phrenic discharge; the horizontal bar indicates pulse timing.

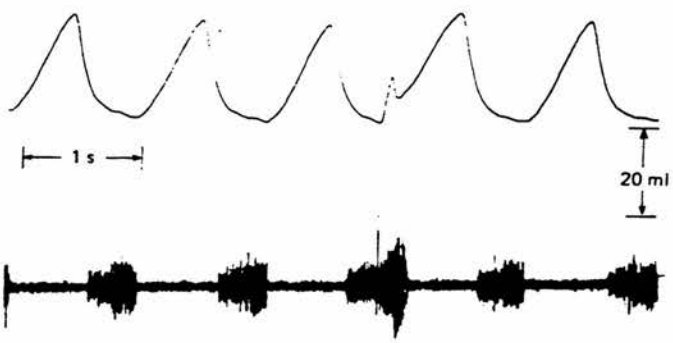

Fig. 6. Effect of a pulse of inflation given during phrenic 'inspiration' in an anaesthetized paralysed artificially ventilated rabbit; the horizontal bar indicates pulse timing.

Phrenic responses to pressure pulses in paralysed rabbits could be repeated in consecutive cycles, i.e. there was no refractoriness. In the paralysed animals only three phrenic discharges with patterns characteristic of an augmented breath were seen. They seemed to occur during the rare occasions when phrenic discharge was coincident with peak inflation volume.

DISCUSSION

In spontaneously breathing rabbits the inspiratory augmenting responses to pressure pulses were generally indistinguishable from spontaneous augmented breaths. Only the subsequent decrease in T_E was more consistent in the triggered responses. Consequently we shall refer to 'spontaneous' and 'triggered' augmented breaths. Our method of measuring end-tidal P_{CO_2} and the absence of a method of rapidly following blood-gas tensions prevented us from observing any transient changes in these variables. Whether or not pulses of inflation and deflation caused transient alterations in blood–gas tensions, the changes in arterial chemoreceptor activity that might result would not occur until one or two breaths later (Leitner, Pagès, Puccinelli & Dejours, 1965) and could not have influenced the characteristics and generation of the triggered augmented breaths. Since the latter were not seen when pulse pressure was set to zero or after vagotomy they were presumably due to excitation of lung receptors (see below and Introduction). Our few experiments with single-fibre recording are incomplete (for example the receptors were not localized or fully characterized) and were only intended to test receptor activity in our experimental conditions.

Of the known lung receptors, pulmonary stretch endings seem unlikely to be the agent. Firstly, pulses of either inflation or deflation in inspiration triggered augmented breaths similar in all respects, while recordings from stretch fibres showed that they were excited only by pulses of inflation. Their excitation in inspiration would decrease T_I. Furthermore, block of stretch receptors by SO_2 did not suppress triggered augmented breaths, although they were less frequently produced.

With regard to type J receptors (Paintal, 1969) and C fibre endings (Coleridge & Coleridge, 1977), we have not directly tested their activity during pulses. However, in cats inflations of up to four times tidal volume fail to excite J receptors (Paintal, 1969), and in rabbits they are not stimulated, or only very weakly, by large maintained inflations or deflations (Sellick & Widdicombe, 1970).

Lung irritant receptors in rabbits are strongly excited both by inflation and deflation (Mills, Sellick & Widdicombe, 1969; Sellick & Widdicombe, 1970). They are not blocked by SO_2 inhalation (Davies, Dixon, Callannan, Husczuk, Widdicombe & Wise, 1978). We therefore agree with Sellick & Widdicombe (1970) and Glogowska *et al.* (1972) that rapidly adapting lung 'irritant' receptors are responsible for augmented breaths. The capacity of pulses in one respiratory cycle to affect subsequent cycles has been described by us (Davies & Kohl, 1979) and by Karczewski, Budzinska, Gromysz, Herczynski & Romaniuk (1976) for a slightly different situation. We cannot say whether this effect is due to a central neural delay or some other factor, but the similarity of our results to those of Karczewski who produced his effects by electrical stimulation suggests the involvement of vagal pulmonary receptors.

In spontaneously breathing animals the triggered augmented breath was 'all-or-none'. In paralysed rabbits, the bursts of phrenic discharge triggered by pulses given during the phrenic pause were also all-or-none, with mean duration 0·31 s or 0·6 times the control T_I. The graded increase in T_I produced by pulses given during phrenic activity can be explained by the introduction with a very short latency, of such a burst of phrenic activity; i.e. the increase in T_I depended on the overlap of

the spontaneous phrenic activity and the stimulated burst. If an augmented breath in a spontaneously breathing rabbit were due to a normal phrenic discharge (0·41 s duration) followed immediately by a reflexly induced discharge of 0·31 s, the T_I of the augmented breath would be 1·75 times contol. This value is close to that observed experimentally (1·85 ± 0·22). This description of an augmented breath could explain both the all-or-none and the biphasic characteristics of the augmented response.

In paralysed rabbits pulses of deflation given during phrenic inactivity triggered bursts of phrenic discharge with very short latencies. Pulses of inflation given at the same phase produced bursts of phrenic activity at the time of the next peak of inflation volume; this would correspond to the peak of irritant receptor discharge induced by the pump inflation. Our hypothesis is that a deflation pulse during phrenic inactivity (i.e. early during lung inflation) is a more powerful stimulus to lung irritant receptors than an inflation pulse; the latter is only able to provoke phrenic discharge when the induced afferent activity is added to that caused by lung inflation by the pump. This hypothesis has yet to be tested, but it could explain why in spontaneously breathing animals any augmentation of phrenic activity always occurred at the end of a normal inspiration (and therefore peak tidal volume) independent of the timing of the pulse. However it would not explain why, in spontaneously breathing rabbits, pulses were less effective in triggering augmented breaths when given towards the end of inspiration (see below).

One of the striking features of our results was the existence of a refractory period after an augmented breath. A similar refractoriness was reported by Reynolds (1962) after the augmented breaths produced by the ventilation of cats with large tidal volumes, and by Glogowska *et al.* (1972) after augmented breaths triggered by chemoreceptor stimulation in cats and rabbits. These authors did not describe whether the refractoriness they produced affected T_I and T_E or whether, as in this study, it was restricted to T_I. The refractory period lasted for 1–2 min in our experiments. Although spontaneous irritant receptor discharge was decreased during this period, as found by Sellick & Widdicombe (1970), irritant receptors were still excited by pulses of inflation and deflation. Refractoriness was absent during paralysis. This may indicate that it is not a purely central mechanism, following an extended inspiration.

It is impossible to say whether the lack of response to a pulse in inspiration sometime after an augmented breath was due to this refractoriness or some other factor. We therefore have no clear base line against which to measure refractoriness and restrict ourselves to reporting its presence.

Pressure pulses were less able to produce an augmented breath during the second half of inspiration; however, the irritant receptors were more sensitive to inflation pulses and less sensitive to deflation pulses during this time. We must conclude that, towards the end of a spontaneous inspiration, irritant receptor activity is prevented from causing an augmented breath. It follows that a spontaneous augmented breath must be caused by the summation of the inspiratory drive with the reflex effects of irritant receptor activity early in inspiration, at a time when the receptors would be sensitized by collapse of the lungs (Sellick & Widdicombe, 1970).

Respiratory frequency increased for a few breaths following an augmented breath. During this period activity of arterial chemoreceptors was probably reduced by a

transient increase in arterial P_{O_2} (Biscoe & Purves, 1967). However, transient reduction in chemoreceptor activity by inhalation of a few breaths of oxygen decreases both tidal volume and respiratory frequency (Leitner *et al.* 1965) and cannot account for the acceleration of respiration reported here. Reduction of airways P_{CO_2} after an augmented inspiration may increase activity of CO_2 sensitive stretch receptors, but this would also be expected to cause an increase in T_E. It is therefore possible that the effects of irritant receptor stimulation during an augmented breath extends over a few respiratory cycles.

Pulses of deflation in expiration always decreased T_E. The change in T_E was not altered by block of stretch receptors by SO_2. Pulses of inflation early in expiration sometimes shortened T_E by as much as that due to pulses of deflation. Later in expiration pulses of inflation produced either less shortening or lengthening of T_E (Fig. 2). Block of stretch receptors reversed any lengthening to a shortening. Our results are consistent with the view that excitation of irritant receptors during expiration shortens T_E; and that if stretch receptors are simultaneously excited they could dominate the response and lengthen T_E. D'Angelo (1978), using a differential d.c. block of the vagus, demonstrated a 'shortening effect on T_E exerted by rapidly adapting or "irritant" receptors'.

Knox (1973) used deflation pulses throughout expiration in cats, and observed shortening of T_E with constant latency except for a graded effect with pulses early in expiration (Fig. 8 in Knox, 1973). He did not observe any shortening of T_E with pulses of inflation, possibly because he used 200 ms pulses which would stimulate stretch receptors more than our 100 ms pulses. The response to a pulse presumably depends on the relative balance of excitation of stretch and irritant receptors.

A pulse of deflation given late in expiration occasionally produced an augmented breath in addition to shortening T_E. With these exceptions, when a pulse shortened T_E the following T_I was shortened, whether or not stretch receptors were blocked. This shows that T_I need not be independent of the previous T_E, as suggested by Clark & von Euler (1972) and Knox (1973). Karczewski *et al.* (1976) reported that electrical stimulation of the vagi in bilaterally vagotomized rabbits produced a smaller decrease in T_I than in T_E, and that the changes in T_E preceded the changes in T_I by one respiratory cycle.

We conclude that irritant receptor stimulation during inspiration produces an augmented breath followed by a refractory period for such breaths; their stimulation in inspiration or expiration shortens expiration without refractoriness.

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Effect of Pulses of Pressure Applied to the Larynx of Rabbits on Their Pattern of Breathing

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Abstract. Brief pulses of positive or negative pressure were applied in inspiration or expiration to the lumen of the larynx of anaesthetised rabbits. The reflex response depended on the nature of the pulse and its position in the respiratory cycle. Pulses in inspiration suppressed and then extended phrenic activity. Pulses in expiration shortened that expiration. Changes in duration of inspiration were independent of tidal volume. Activity of afferent fibres in the superior laryngeal nerve suggested the origin of these reflexes was receptors in the joints of the larynx. Coughs or the aspiration reflex were never provoked. Our results suggest a reflex role for the larynx in control of quiet breathing and an influence on the mechanisms relating inspiratory duration to tidal volume.

Key words: Larynx – Breathing – Reflexes – Receptors

Introduction

Protection of the airways is an important and perhaps the phylogenetically oldest function of the larynx [18]. Cough, expiratory reflex and apnoea after stimulation of laryngeal mucosa [1, 14, 15, 22] apparently serve this purpose. The slowing and deepening of breathing described by Boushey et al. [1] as a consistent late change after stimulation of the larynx is difficult to classify as protective. This suggests that the larynx could play some reflex role in the regulation of the normal pattern of breathing, provided there was an adequate physiological stimulus. The most likely stimulus is pressure changes within the larynx during the respiratory cycle. We therefore investigated the influence of brief pulses of pressure to the larynx on the pattern of breathing.

Methods

We used 26 adult New Zealand White rabbits, weighing between 2.4 and 4.1 kg. Not all tests were carried out on all rabbits. Anaesthesia was induced and maintained with sodium pentobarbitone (Sagatal, May and Baker, 40 mg/kg). Polyethylene catheters were tied into the femoral vein and artery. The rabbits breathed spontaneously through a cannula in the lower cervical trachea connected to a Fleisch pneumotachograph No. 0, and differential pressure transducer (Statham PM15E). Air flow signal was electronically integrated to provide a signal proportional to tidal volume. A second cannula was tied in the trachea just below the larynx, its end level with the first tracheal ring. This cannula was connected, by means of solenoid operated valves, either to atmosphere or a large drum (20 l) maintained at the required pressure. Connection of the larynx to the drum was usually made for 200 ms by a Digitimer which operated the valves. A third cannula was tied into the larynx from above and used for measuring intralaryngeal pressure with a Micromanometer (Furness Controls Ltd.). The oesophagus was tied off. Phrenic activity was recorded from multifibre strands of single fibres of the upper cut root of right phrenic nerve placed in a through of liquid paraffin. Action potentials were picked up by two platinum electrodes and amplified by a Palmer 8121 preamplifier. Phrenic activity simultaneously started a Digitimer (3290 Devices) which operated the valve system providing pressure pulses of precise duration and delay after the beginning of phrenic activity. The duration of the pulse and its timing could be altered. Activity of single fibres was recorded from the cut distal end of the right SLN by the method used with the phrenic nerve. The experimental set up is shown in Fig. 1. Variables were recorded by an ultraviolet recorder (SE 600) and simultaneously by a seven channel tape recorder (RACAL, Store 7D).

Duration of inspiration and expiration were measured from the recording of phrenic and flow signals. Duration of inspiration was considered to be the time from the beginning of phrenic activity to its rapid decline. Duration of expiration was the remainder of the respiratory cycle. While recording from single phrenic fibres we

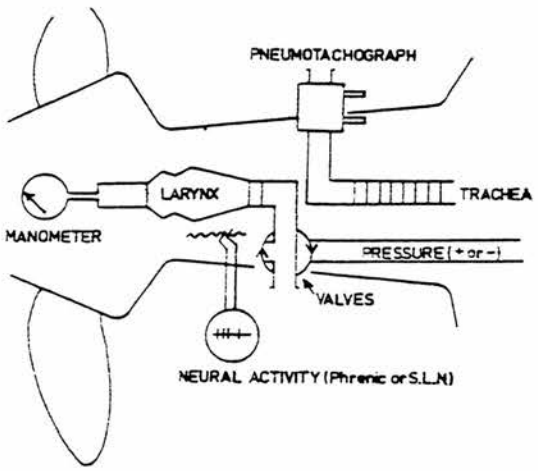

Fig. 1. Experimental set-up

used inspiratory and expiratory air flow to define inspiration and expiration. At the beginning of an experiment we tested the response of the rabbit to a pulse of +40 cm water applied during inspiration. Out of 26 rabbits three responded by a prolongation of inspiration of less than by 10%. These three were not included in further tests.

Results are expressed as mean ± SE. Student's *t*-test was used for statistical evaluation of results; $p < 0.05$ was considered as significant.

Results

Unless given in absolute terms (ms) duration of inspiration (t_I), duration of expiration (t_E) and tidal volume (V_T) for the breath containing the pulse are expressed relative to the corresponding value in the previous breath. This means that an increase is represented by values higher than 1, decrease lower than 1. Variability of control values was tested by expressing the control breath relative to the previous breath in each run. This method of treating results standardized the frequencies of breathing of different animals under control conditions. Absolute values of control t_I varied from 400 to 720 ms (564 ± 15 ms), t_E 1020 to 2060 ms (1329 ± 71 ms).

Pulses During Inspiration

Pulses of negative pressure from −10 to −40 cm water applied during inspiration failed to change the pattern of breathing. The mean relative value of t_I after a pulse of −40 cm water was 1.03 ± 0.02, t_E 0.99 ± 0.03 and V_T 0.99 ± 0.02.

Application of a pulse of positive pressure to the larynx during the first half of inspiration resulted in a decrease of phrenic activity which then resumed and was prolonged (Fig. 2). The following expiration was shortened. The higher the pressure of the pulse the greater prolongation of t_I (Fig. 3). Fig. 4 shows the response of a single phrenic fibre to a pulse of +40 cm water. There was a short pause in firing after the pulse, but the total number of spikes was greater than during control inspiration. Of 31 phrenic fibres 19 responded to a pulse of pressure by a pause in firing and then prolongation of firing, ten by prolongation of firing only, two did not respond. In all cases V_T of the pulsed breath was not significantly different from control value.

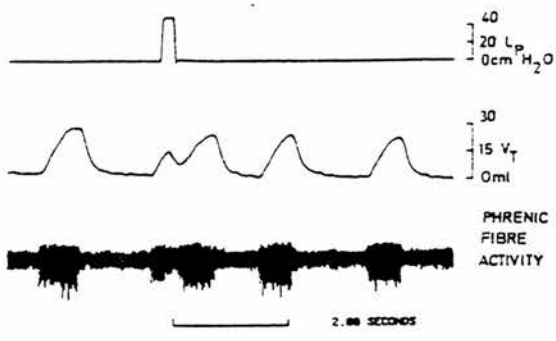

Fig. 2. Effect of a pulse of positive pressure applied to the lumen of the larynx during inspiration. Traces from above down. *Lp* – laryngeal pressure. V_T – tidal volume. Phrenic activity

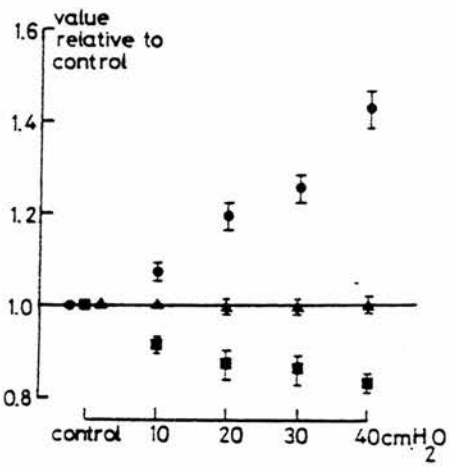

Fig. 3. Effect of 200-ms pulse of different positive pressures applied to the lumen of the larynx 300-ms after the start of inspiration. ●=t_I; ▲=V_T; ■=following t_E (mean±S.E. 40 tests, 15 rabbits)

The opposite signs of the changes in t_I and t_E resulted in unchanged cycle duration. Control $t_I = 564 \pm 15$ ms, $t_E = 1329 \pm 54$ ms, cycle = 1893; pulse of +20 cm H_2O $t_I = 669 \pm 22$ ms, $t_E = 1207 \pm 63$ ms, cycle = 1876; $n = 40$. Despite t_I being prolonged from control 564 ± 15 ms to 669 ± 22 ms after +20 and to 771 ± 25 ms after +40 cm water pulses, corresponding values of V_T were 21.8 ± 0.6 and 21.8 ± 0.6 ml, identical with control values.

The timing of the pulse in inspiration was important. In 14 experiments (five rabbits) we applied a pulse of +40 cm water to the larynx at 10%, 20%, 40%, 60% and 80% of control t_I. Pulses applied during the first 60% of t_I were effective (t_I 1.23 ± 0.05 at 10%, 1.33 ± 0.05 at 20%, 1.31 ± 0.07 at 40% and 1.33 ± 0.06 at 60%). Pulses applied at 80% of t_I did not change t_I (1.01 ± 0.03). The following t_E was always shortened (0.85 ± 0.04 at 10%, 0.82 ± 0.05 at 20%, 0.86 ± 0.03 at 40%, 0.83 ± 0.03 at 60% and 0.85 ± 0.05 at 80% of t_I).

Pulses During Expiration

Pulses of positive pressure applied during expiration shortened that expiration (Fig. 5). The relation between pressure of pulse and shortening of t_E is shown in

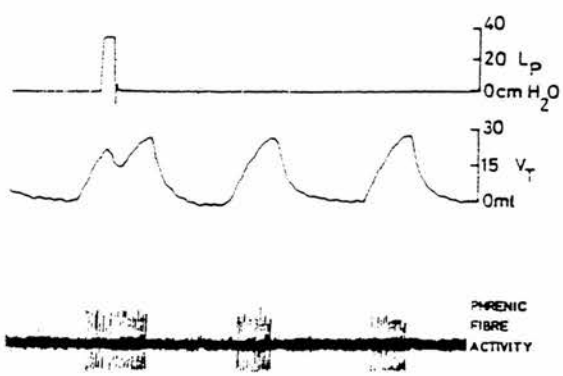

Fig. 4. Effect of a pulse of positive pressure applied to the lumen of the larynx on the activity of a single phrenic fibre. Traces as Fig. 2

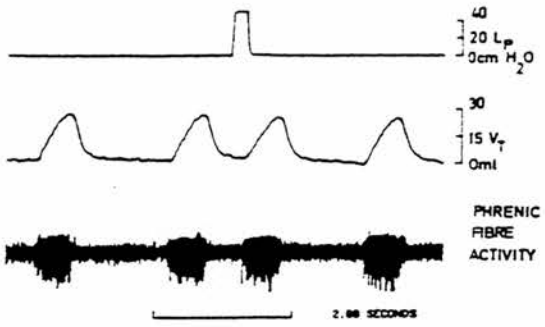

Fig. 5. Effect of a pulse of positive pressure applied to the lumen of the larynx during expiration. Traces as Fig. 2

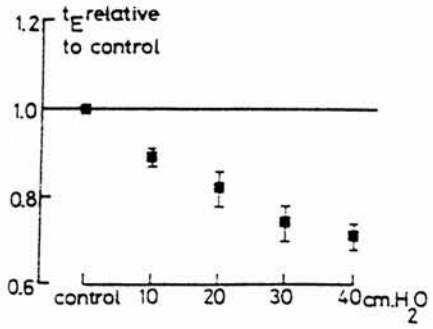

Fig. 6. Effect of 200-ms pulse of different positive pressures applied to the lumen of the larynx during expiration on the duration of that expiration (mean ± S.E. 30 tests, 15 rabbits)

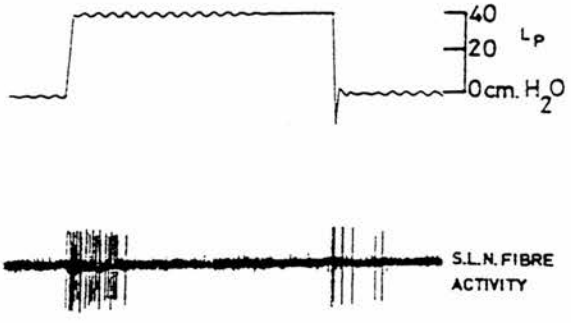

Fig. 7. Response of one type of superior laryngeal nerve fibre to a 800-ms, 40-cm H_2O positive pressure pulse applied to the lumen of the larynx. Traces from above down. Lp = laryngeal pressure. Activity of fibre

Fig. 6 for pulses given at 20% t_E. Pulses of negative pressure during expiration failed to change the pattern of breathing.

Cutting both SLN and recurrent nerves bilaterally abolished the effect of pulses ($n=8$). Filling the larynx with local anaesthetic (4% w/w lignocaine hydrochloride) in two rabbits did not completely block the response to the pulse. We found prolongation of t_I by 15% after pulses of +40 cm water in t_I during the first 3 min after administration of the local anaesthetic.

We recorded activity of 73 single fibres from the SLN (eight rabbits). According to their spontaneous activity these fibres could be divided into two groups: fibres with regular spontaneous activity and fibres with no or little spontaneous activity.

The activity of either type of fibre was only significantly modified by pulses of positive pressure.

Fibres from the first group responded by accelerating firing (22 fibres) or by ceasing firing (6 fibres). Fibres with little or no spontaneous activity responded with activity throughout a pulse (7 fibres) or with bursts of activity at the onset and the offset of a pulse (38 fibres) (Fig. 7).

Discussion

Pressure within the larynx of a normally respiring animal is negative during inspiration and positive during expiration. We produced no changes in pattern of breathing by pulses of negative pressure applied during inspiration. The role of pressure stimulation during inspiration is minimal. During expiration a role for laryngeal reflexes seems more likely. The pressure which we used to shorten expiration seems high in comparison to 2 cm water reported by Remmers and Bartlett [19] in spontaneously breathing cats, but Harding, Johnson and McClelland [11] recorded intratracheal pressure up to 10 cm water in conscious lambs. Furthermore pressure of +5 cm water reduced expiration to 0.91 ± 0.02 of control value. Removal of laryngeal stimulation could play a role in the slight prolongation of expiration after opening of a tracheostomy during expiration [19] as well as the feedback mechanisms originating in extrathoracic tracheal receptors suggested by these authors.

It must be borne in mind that our rabbits were anaesthetized and there was no flow through the larynx. Anaesthesia has considerable effect on respiratory reflexes, depressing cough [15] and enhancing the Hering-Breuer inflation reflex [3].

The difference between electrical stimulation of SLN and stimulation of larynx by brief pulses of pressure is not surprising. Shortening of t_E after pulses in expiration (compared to prolongation of t_E after electrical stimulation of SLN [16]) can be explained by the very different character of the stimulation. Synchronous activation of all the fibres of the SLN by an electrical stimulus is probably less physiological than changes in intraluminal pressure.

The difference between the reflex we describe and those described by others after mechanical and chemical stimulation of laryngeal mucosa are obvious. Cough [1, 15] and expiratory reflex [14] include expiratory efforts which we never saw. Apnea, as described by Boushey et al. [1], lasted for more than three breathing cycles. Slowing and deepening of breathing implies increase in V_T and prolongation of cycle duration, neither of which we found. The nature of the stimuli used, touching of laryngeal mucosa by filaments or cotton wool pledget or exposing the surface of the larynx to different chemicals, is probably responsible for these differences.

The inability of topically applied local anaesthetic to abolish the response to a pulse of pressure suggests a role of deeper receptors. Although receptors are present in laryngeal muscles the most important proprioceptive mechanism in the larynx is probably that of the position receptors [13]. This could explain the difference in sensitivity to positive and negative pressure. The anatomical structure of the larynx probably allows larger changes of configuration (and wall tension which is probably what affects receptor activity) in response to positive rather than negative pressure.

Single fibre activity in the superior laryngeal nerve confirmed the classification of Boushey et al. [2]. We could not correlate any fibres with the particular types of

laryngeal receptors described by Storey [20]. However one group of afferent fibres showed a distinct on and off response (Fig. 7).

The diametrically opposite effects of phrenic inhibition followed by phrenic prolongation may be the result of stimulation of different types of receptors. Lack of effect of a pulse after 80% of inspiration could be the result of changes of central sensitivity. On the other hand prolongation of phrenic activity could be a reflex response to the change of the course of inspiration due to a decrease of phrenic activity after the pulse. Cross, Jones and Guz [8] recently showed showed that changing the shape of inspiration always resulted in prolongation of phrenic discharge of that inspiration.

The changes of t_I and t_E when the pulse was applied in inspiration supports the idea of at least partly independent regulation of t_E. Shortening of t_E was not only the passive result of the prolongation of t_I in a given cycle duration (t_I occupying more of the cycle of fixed duration), because the pulse applied at 80% of t_I shortened the following t_E despite not changing t_I. Stimulation of the larynx by pressure thus always shortened t_E. This is similar to stimulation of lung irritant receptors described by Davies et al. [10].

Clark and von Euler [7] and Bradley et al. [4] have suggested that in cats t_I is terminated by increasing pulmonary stretch receptor activity reaching a central threshold which decreases with time. Once this threshold is reached an inspiratory off switch is triggered. Applying the figures obtained in rabbits by D'Angelo and Agostoni [9] and by Trenchard [21] to this model we would expect prolongation of t_I to the degree we obtained to terminate inspiration at 50% of the control value of V_T. This was not the case. V_T reaching control values despite prolongation of t_I. Electrical stimulation of the vagi, below the threshold required to terminate t_I, can produce a reduction in inspiratory flow and prolongation of t_I [5]. No mention of the V_T associated with such a change was made by these authors. Younes et al. [23] produced similar extensions of t_I using transient changes in lung volume. It seems however that, independent of such influences from the lungs, afferent neural activity from the larynx may in some way change the off-switch threshold or attenuate the influence of pulmonary stretch receptor activity in the control of breathing.

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In; Proc. International Union of Physiological Sciences (Sydney) 1983.

322.08

EFFECT OF LUNG INFLATION AND DEFLATION ON PATTERN OF BREATHING IN CONSCIOUS DOGS

A. Davies, J.A. Nadel and Gail Weinman C.V.R.I., University of California, San Fransisco.

Brief (400ms) and sustained (several breaths) inflating and deflating pressures were applied to the lungs of conscious dogs before and during differential cold block (7°C) of their cervical vagi. Sustained positive pressure approximately doubled end inspiratory volume but never caused significant reduction of inspiratory duration (T_I). The associated increase in expiratory duration (T_E) was abolished by vagal cooling. Sustained negative pressure also produced no significant change in T_I but a significant reduction in T_E. However T_I could be terminated by rapid increases in lung volume with vagi warm or cool. Apart from during augmented breaths T_I was much less susceptable to change by experimental procedures than was T_E. There did not appear to be a T_I terminating mechanism sensitive to the sustained changes in lung volume we produced in these conscious dogs.

Publication 34.

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1984, *20*, 43-47

AIRWAY RECEPTORS IN COUGH

ROLE DES RÉCEPTEURS DES VOIES AÉRIENNES DANS LA TOUX

G. Sant'Ambrogio *, F.B. Sant'Ambrogio *, A. Davies **

ABSTRACT : Sulphur dioxide inhalation (200 ppm) suppresses the inflation apnea in rabbits, but not in dogs. In rabbits, SO_2 blocks airway slowly adapting stretch receptors (SAR) while rapidly adapting irritant receptors (RAR) remain largely unaffected. We studied cough elicited by mechanical irritation of the extrapulmonary airways and larynx in 13 rabbits and 4 dogs, anesthetized and spontaneously breathing, before and after SO_2 inhalation. In rabbits, once SO_2 had blocked the Hering-Breuer inflation reflex, mechanical stimulation of the trachea and carina failed to elicit cough ; laryngeal stimulation was still effective though the response was attenuated. In dogs, SO_2 inhalation (even up to 1,000 ppm) did not abolish either inflation apnea or cough to tracheobronchial mechanical stimulation. We recorded the response to mechanical probing of tracheal RAR, before and after SO_2 exposure, in 6 rabbits. We found that the activity of these RAR was still present after cough and inflation apnea had disappeared. These results suggest a significant role of slowly adapting airway receptors in the cough reflex.

Airway receptors ; cough ; sulphur dioxide ; vagus nerves.

Cough is a reflex act initiated by appropriate chemical or mechanical stimulation of the larynx and the more proximal portion of the tracheobronchial tree. It involves several groups of muscles activated in a coordinated pattern to produce a deep inspiration followed by a strong expiratory effort initially against a closed larynx which then suddenly opens releasing a strong blast of air through the airways [10]. Cough is generally thought to be initiated through the stimulation of mucosal endings, located in the larynx and trachea down to its bifurcation, functionally identifiable by a very rapidly adapting discharge in response to mechanical stimulation [13].

In the course of experiments in which we utilized SO_2 to produce a selective block of airway slowly adapting stretch receptors (SAR) in rabbits [7, 8], we noticed that the rabbits did not cough in response to mechanical irritation of the trachea and coughed less frequently in response to mechanical stimulation of the larynx. Considering the selective blocking action of SO_2 on stretch receptors [6], these observations seem to imply an important role of these endings in the tussive response. BUCHER [2] and BUCHER and JACOT [3] had previously proposed a role of airway stretch receptors in the mechanisms of cough, although some of their evidence was based on a supposedly selective action of benzonatate [nonaethylene-glycol-monomethyl-ether (b-n-butylamino) benzonatate] on these endings which has since been shown to be untenable [11].

The blocking action of SO_2 has been found to be specific to SAR with rapidly adapting irritant receptors (RAR) remaining largely unaffected [6]. Moreover, reflex responses evoked by pneumothorax or injection of histamine, attributable to RAR, were also preserved [6]. However, the study of DAVIES *et al.* [6] did not require a precise anatomical location of the RAR investigated and we cannot exclude the possibility that some of the RAR, located in the trachea or around the carina, *i.e.* those usually thought of as being responsible for cough, might be blocked by SO_2. Thus the possibility exists that the absence of cough in rabbits exposed to SO_2 depends on the blockade of tracheal RAR while the concomitant abolition of the Hering-Breuer inflation apnea is due to a block of SAR.

This study investigates the effect of blocking the activity of airway slowly adapting stretch receptors, as indicated by the elimination of the Hering-Breuer inflation apnea, on cough in response to mechanical stimulation of the larynx and the trachea in rabbits. Moreover the action of SO_2 on tracheal RAR was specifically evaluated to test any effect of this gas on these receptors. The study provides supportive evidence for a role of airway stretch receptors in the genesis of coughing.

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METHODS

Cough experiments

Experiments were performed on thirteen New Zealand White rabbits weighing between 2.0 and 3.2 kg and on four mongrel dogs weighing between 11 and 16 kg. The

rabbits were anesthetized with intravenous sodium pentobarbital (40 mg · kg^{-1}) and the the dogs with a mixture of chloralose (0.1 g · kg^{-1}) and urethane (1.0 g · kg^{-1}). Further anesthetic was administered as necessary via a polyethylene catheter inserted in a femoral vein. Urethane and chloralose anesthesia was used in dogs, instead of sodium pentobarbital, to obtain a more regular cough response to the mechanical stimulation of the trachea. Pentobarbital was preferred in rabbits for the ease of its initial administration and found quite adequate in allowing regular cough responses following tracheal and laryngeal stimulation. Anyhow similar observations, as described here, were verified in the course of an other study in rabbits anesthetized with either chloralose-urethane or urethane. Systemic arterial blood pressure was monitored by a Statham pressure transducer connected to a catheter in a femoral artery. One arm of a T-shaped cannula was inserted into the animal's trachea just below the cricoid cartilage. In rabbits, a polyethylene catheter, filled with saline, was introduced in the esophagus and connected to a pressure transducer used to measure intrathoracic pressure ; tracheal pressure was measured from the side arm of the tracheal cannula by a Statham pressure transducer. In dogs, an esophageal balloon and the side arm of the tracheal cannula were connected to a Statham differential pressure transducer to measure transpulmonary pressure. Electromyograms (EMG) were obtained from the diaphragm and the right external oblique muscle using wire electrodes connected to AC coupled amplifiers. Blood pressure, esophageal and tracheal or transpulmonary pressure, diaphragm and abdominal muscle EMG were displayed on a Gould-Brush chart recorder.

In each animal, the Hering-Breuer inflation apnea and the cough response were first tested. To elicit the Hering-Breuer reflex, the lungs were inflated to 10-15 cmH_2O transpulmonary pressure. To provoke cough, the interior of the trachea or larynx was stroked with the tip of a polyethylene catheter inserted through the tracheal cannula down to the carina and then withdrawn, or through the hole made below the cricoid cartilage up to the vocal folds. Each trial consisted in three consecutive strokes of the catheter. Each animal was challenged with two to six trials. Cough was identified by characteristic pressure changes and the EMG signals.

SO_2 was mixed with air in a Douglas bag and administered as described by DAVIES *et al.* [6] at a concentration of 200-300 ppm in rabbits and 350-1000 ppm in dogs. The animal breathed the gas mixture for 10-20 min or, in rabbits, until the Hering-Breuer inflation reflex disappeared indicating that the slowly adapting receptors were blocked. At this point, the cough challenges were repeated and, in rabbits, repeated again when the Hering-Breuer inflation reflex reappeared.

Nerve recording

To ascertain whether SO_2 affected the tracheal rapidly adapting receptors, their activity was recorded before and immediately after exposure to SO_2 in six rabbits anesthetized and prepared as described above. They were then paralysed with gallamine (5 mg · kg^{-1}) and artificially ventilated. Electrical activity was recorded from multifibre preparations of the central cut end of the upper root of the right phrenic nerve and simultaneously from single nerve fibres originating in tracheal rapidly adapting receptors, dissected out of the peripheral cut end of the right vagus. Both nerves were immersed in paraffin oil and activity was picked up by platinum electrodes. The left vagus nerve was intact. The vagal action potentials were recognized as originating from rapidly adapting mechanoreceptors by their irregular frequency of discharge and rapid adaptation to a maintained stimulus. The tracheal location of these receptors was ascertained by probing the lumen of the trachea with a cuffed catheter. Sulphur dioxide at a concentration of 200-300 ppm was administered through the inlet of the respirator. The abolition of the Hering-Breuer inflation reflex following SO_2 administration could be determined by stopping the ventilator at peak inflation and observing that phrenic activity remained unaffected. A further indication was also provided by observing that the phrenic discharge was not any longer confined to the periods of pump « expiration » [8]. The phrenic coughing to mechanical irritation of the trachea was elicited by probing the trachea through a side arm of the tube which connected the rabbit to the pump. The occurrence of cough was inferred by the alteration in the phrenic discharge. The receptor discharge was evaluated before and after SO_2 administration. Action potentials from rapidly adapting receptors and phrenic nerve activity were recorded on a Visicorder.

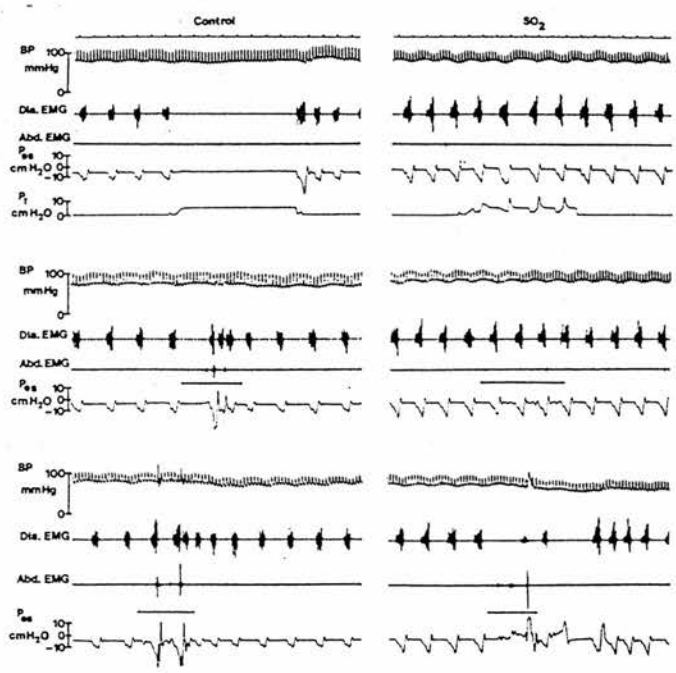

Fig. 1. — Effect of SO_2 administration on the cough reflex in an anesthetized rabbit. In control situation, lung inflation to 10 cmH_2O inhibited breathing (Hering-Breuer inflation reflex). Cough (indicated by increased activity in diaphragm and abdominal muscles) could be elicited by both tracheal and laryngeal stimulation. After SO_2 administration (200 ppm) the Hering-Breuer inflation reflex has disappeared and cough could be elicited only with laryngeal stimulation. Left-hand tracings : before SO_2 administration ; right-hand tracings : after SO_2 exposure. Upper panels : effects of lung inflation ; middle panels : effects of tracheal stimulation ; lower panels : effects of laryngeal stimulation. In each panel, from top to bottom : time in seconds (only in upper panel), arterial blood pressure, diaphragm EMG, abdominal muscle EMG, tracheal or esophageal pressure. Horizontal bars indicate periods of stimulation.

RESULTS

Cough experiments

Figure 1 illustrates a typical experiment. Under control conditions, when inflation of the lungs evokes a typical apnea (Hering-Breuer inflation apnea ; top left hand panel), probing of the trachea and the larynx elicited cough as evidenced by the pattern of diaphragmatic and abdominal muscle EMG activity and by tracheal and esophageal pressure swings (middle and bottom left hand panel). When sulphur dioxide exposure abolished the Hering-Breuer inflation apnea (top right hand panel), tracheal probing failed to cause cough (middle right hand panel), unlike laryngeal probing. However, cough generated from the larynx during SAR block consistently appeared to be weaker (bottom right hand panel). When the Hering-Breuer inflation apnea recovered, 20-60 min after termination of SO_2 administration, cough, in response to mechanical irritation of both trachea and larynx, reappeared and was restored to its original strength.

We performed 49 trials on thirteen rabbits (both before and after SO_2 inhalation), each consisting of a series of three strokes delivered with a catheter to the mucosal surface of the trachea down to its bifurcation. Under control conditions coughing was evoked in 98 % of the trials (fig. 2, top panel). When sulphur dioxide inhalation had abolished the inflation apnea, coughing was evoked in only 2.4 % of the trials (fig. 2, top panel).

Mechanical irritation of the larynx evoked cough in virtually all trials (94.6 %) in control conditions and in 70 % of the trials after SO_2 block of the Hering-Breuer inflation apnea (fig. 2, top panel).

We also carried out 15 trials in four dogs. Figure 3 illustrates the results of one of these experiments. The Hering-Breuer inflation apnea could be obtained both before and after sulphur dioxide exposure (fig. 2, bottom panel ; fig. 3, top panels). Similarly, mechanical stimulation of the tracheal surface was found to be equally effective in provoking cough before and after exposing the dog to sulphur dioxide (fig. 2, bottom panel ; fig. 3, bottom panels).

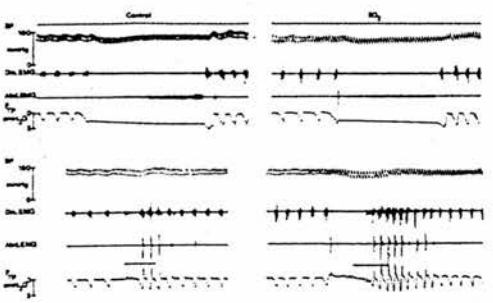

Fig. 3. — Effect of lung inflation (top tracings) and tracheal stimulation in an anesthetized dog (bottom tracings), before (left-hand tracings) and after (right-hand tracings) administration of SO_2. In each set of tracings, from top to bottom : time in seconds (only on top tracing), systemic arterial blood pressure, diaphragm EMG, abdominal EMG and transpulmonary pressure. The Hering-Breuer inflation reflex and cough can be elicited before and after administration of SO_2.

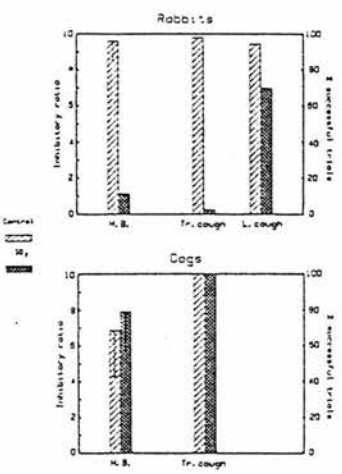

Fig. 2. — Graphical representation of the effect of SO_2 administration (200 ppm) on the Hering-Breuer reflex (HB), on cough elicited by tracheal stimulation (Tr. cough) and on cough elicited by laryngeal stimulation (L. cough). The left-hand ordinate, expressed as inhibitory ratio (ratio between duration of expiration during lung inflation and during control ; an inhibitory ratio = 1.0 indicates absence of HB reflex), applies to the Hering-Breuer inflation reflex (vertical bars indicate standard error) ; the right-hand ordinate applies to the cough trials and it is expressed in percent of trials successful in eliciting cough. Laryngeal stimulation was performed only in rabbits.

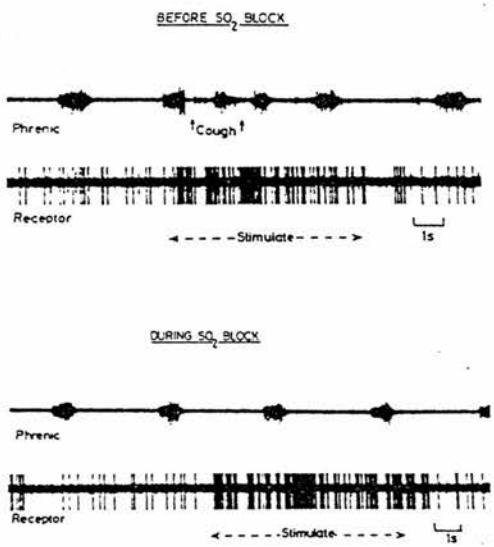

Fig. 4. — Effect of SO_2 on a tracheal rapidly adapting receptor in an anesthetized rabbit. Tracheal stimulation increases the discharge of the receptor before (upper panel) and after (lower panel) administration of SO_2. Cough, as shown by the phrenic ENG, can be evoked only before exposure to SO_2. In each panel, the top tracing represents the phrenic ENG and the bottom tracing action potentials recorded from a fiber originating from a tracheal rapidly adapting receptor.

Recording from tracheal rapidly adapting receptors in the rabbit

Action potentials from six tracheal rapidly adapting receptors, identified and stimulated as described in Methods, were recorded in six rabbits. The response to this mechanical stimulation was obtained before and after a period of SO_2 inhalation sufficient to block inflation apnea. No consistent changes were noticed in the activity evoked from tracheal rapidly adapting receptors by the probing procedure before or after sulphur dioxide exposure (fig. 4).

DISCUSSION

Sulphur dioxide inhalation has been shown to provide, at least in the rabbit, a selective block of airway slowly adapting stretch receptors, rapidly adapting receptors being only transiently stimulated by this gas. Probing the trachea with a catheter provides an adequate stimulus to rapidly adapting receptors, but does not result in any direct stimulation of slowly adapting stretch receptors. A possible excitatory effect on these endings might be introduced secondarily through a reflex contraction of airway smooth muscles due to the stimulation of rapidly adapting receptors, but this effect would not be expected to be either prompt or marked. It was therefore surprising to find that coughing in response to mechanical irritation of the trachea was absent when inflation apnea (one index of slowly adapting stretch receptor function) had been abolished, while other reflex responses attributable to rapidly adapting receptors, such as those evoked by negative pressure breathing, pneumothorax and histamine, are known to be still present [6-8].

These findings raise the possibility of a direct involvement of slowly adapting receptors in coughing. Are these receptors a primary agent in the reflex act of coughing ? Why do we not obtain a cough response when indications point to a normal mechanical sensitivity of rapidly adapting receptors ? Indeed, we have found that even those rapidly adapting receptors which for their location in the trachea are expected to be particularly exposed to SO_2 inhalation remained unaffected (fig. 4). Moreover, in the dog, where SO_2 does not affect stretch receptors, coughing in response to stimulation of the tracheal mucosa is fully preserved.

We could hypothesize that sulphur dioxide in the rabbit blocks other types of receptors, perhaps C-fibre receptors, that may also be involved in the cough response. C-fibre receptors have in fact been described in the airways and shown to be activated by local probing of the luminal surface [4]. These receptors could, perhaps concurrently with rapidly adapting receptors, be necessary to initiate the act of coughing. Against this possibility are the observations that coughing is abolished by a selective cold block of vagal conduction which supposedly preserves C-fibre function [12] and, at least in dogs, SO_2 has been found to stimulate, not block, C-fibre receptors [5]. It seems unlikely that SO_2 blocks coughing through a central effect, after being absorbed into the blood, considering its ineffectiveness in dogs in which even higher concentrations of SO_2 have been used.

Considering that cough is essentially an expiratory act, another possibility would involve the role that slowly adapting stretch receptors play in the recruitment of expiratory muscles. It is recognized that whenever stretch receptors are stimulated (by positive pressure breathing or by postural changes) there is an activation of expiratory muscles [1] ; this expiratory recruitment can be abolished by blocking the slowly adapting stretch receptors [8]. It seems therefore reasonable to propose that the role of slowly adapting stretch receptors in coughing is strictly related to their excitatory action on the expiratory muscles. Mechanical irritation of the airways is capable, even after SO_2 exposure, of activating rapidly adapting receptors, but these endings cannot, by themselves, cause cough : presence of stretch receptor activity could play a crucial permissive role in this reflex response through its facilitatory influences on the muscles of expiration. However, this explanation must be accompanied by the caveat that even the inspiratory efforts made by our rabbits in association with a cough were abolished by stretch receptor block. It seems more likely therefore that the cough suppressing action of pulmonary stretch receptor block is associated with the central suppression of afferent information necessary for the initiation or coordination of the complex reflex activity of cough.

Laryngeal coughing is also impaired by SO_2 exposure as was also found [9] in the case of chemical irritation, although not abolished as that from the trachea : the partial persistence of the response could depend on a stronger afferent inflow from the laryngeal luminal surface, not requiring any facilitatory influence from other sources and/or on some differences in the neural connections of the laryngeal afferents.

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RÉSUMÉ : L'inhalation de bioxyde de soufre (SO_2, 200 ppm) supprime l'apnée en inflation (réflexe de Hering-Breuer) chez le lapin, mais pas chez le chien. Il a été démontré antérieurement, chez le lapin, que les récepteurs à la tension des voies aériennes qui s'adaptent lentement (SAR) sont bloqués par le SO_2, alors que les récepteurs à l'irritation qui s'adaptent rapidement (RAR) ne sont que très peu touchés. La toux produite par irritation mécanique des voies aériennes extrapulmonaires et du larynx a été étudiée chez 13 lapins et 4 chiens, anesthésiés et en respiration spontanée, avant et après inhalation de SO_2. Chez le lapin, une fois que le réflexe d'inflation d'Hering-Breuer est bloqué par le SO_2, la stimulation mécanique de la trachée et de la carène ne parvient pas à provoquer la toux ; la stimulation du larynx reste efficace, mais de façon atténuée. Chez le chien, l'inhalation de SO_2 (jusqu'à des doses de 1000 ppm) n'abolit ni l'apnée en inflation, ni la toux par stimulation mécanique trachéobronchique. Nous avons enregistré la réponse à l'exploration mécanique des RAR trachéaux, avant et après exposition à SO_2, chez 6 lapins. L'activité de ces RAR subsiste après que la toux et l'apnée d'inflation ont disparu. Ces résultats suggèrent un rôle significatif des SAR dans le réflexe de toux. Si on considère que la toux est essentiellement un acte expiratoire, on peut suggérer que le rôle des SAR dans la toux dépend de leur influence sur les muscles expiratoires. La persistance partielle de la toux laryngée après exposition au SO_2 peut refléter une action afférente plus forte à partir de l'orifice glottique et/ou certaines différences dans les connections neuronales.

Publication 35.

Davies,A.,Janse,J. & Reynolds,G.W.(1984)

Acupuncture in relief of respiratory arrest.

N.Z. Vet. J. 32, (7), 109-110.

Acupuncture in the relief of respiratory arrest

A. Davies,*† J. Janse,* and G.W. Reynolds*

ABSTRACT

The claim the acupuncture point Jen Chung can be use to stimulate respiration was tested in five anaesthetised sheep, to determine if acupuncture was more effective than a more general nociceptive stimulus in reversing respiratory arrest. Needling this point proved significantly more effective ($P < 0.05$) than needling the tibial nerve in stimulating breathing after respiratory arrest due to halothane. This technique may provide a useful method of reversing respiratory arrest under clinical conditions.

INTRODUCTION

Acupuncture has been proposed as beneficial in a number of clinical situations. It has been claimed that stimulation of specific acupuncture points with needles can restore spontaneous breathing and improve respiration in comatose patients[1] and animals under surgical anaesthesia.[2] However, it is well known that painful stimuli affect breathing, at least in conscious subjects, and it may have been that the respiratory stimulating effects of acupuncture were due to the general arousal caused by a painful stimulus.

The present study was undertaken to see if needling the acupuncture point Jen Chung (Governor Vessel 26) on the nasal philtrum, described as a revival point,[3] was more effective in restoring breathing after respiratory arrest due to an overdose of Halothane (Imperial Chemical Industries) than a more general painful stimulus.

METHODS

Five sheep in which anaesthesia was induced with intravenous Saffan (Glaxo) were intubated and maintained on 2.0–2.5% Halothane. A femoral artery was cannulated and arterial blood pressure monitored using a pressure transducer (Statham Ltd.). Respiratory air-flow was measured using a Fleish pneumotachograph (Gould Ltd.) and electronically integrated to provide a record of tidal volume.

After allowing at least one hour for the effects of the Saffan to wear off, we increased the inhaled Halothane concentration to 6.0–8.0% which produced respiratory arrest. Each animal would spontaneously recommence breathing after a fairly uniform time and so each was allowed a short fixed period of arrest, less than this value, before needling commenced. This period was determined before needling, depended on the individual respiratory drive, and ranged between 10 and 60s.

The three stimuli to breath were

- Needling the acupuncture point Jen Chung (GV26) on the nasal piltrum.
- Needling the tibial nerve.
- Allowing spontaneous respiratory drive due to the animals apnoea to build up without needling.

In the first two procedures a 26 gauge hypodermic needle was introduced to a depth of 25mm and rotated in the prescribed acupuncture manner.[3]

The time taken to restore spontaneous breathing was recorded as the latency from the insertion of the needle to the time the first inspiratory effort was made. The time taken for respiration to spontaneously recommence after arrest due to Halothane and without needling was taken as an index of the animals respiratory drive.

Immediately on recommencement of breathing the Halothane concentration was reduced to the maintenance level (2.0–2.5%). We allowed five minutes at this level of anaesthesia between experimental runs. Each stimulus was repeated three times in random order in each animal.

RESULTS

The results of our experiment on five sheep are given in Table I.

Stimulation of GV26 was significantly more effective than stimulation of the tibial nerve ($P < 0.05$) or no stimulation ($P < 0.001$) in initiating breathing. Stimulation of the tibial nerve had no significant effect ($P > .3$) on the initiation of breathing.

DISCUSSION

Acupuncture point GV26 (Jen Chung) is located in the philtrum level with the lower edge of the nares.[3] Stimulation of this point has been used to enhance breathing in comatose patients, some of which were apnoeic at the beginning of therapy,[1] and in animals being clinically treated with a general anaesthetic.[2] Because of their therapeutic nature these studies were not well controlled in terms of differentiating between specific respiratory stimulation and general arousal that can be caused by a nociceptive stimulus applied to a subject whose central nervous system is not too deeply depressed by trauma or general anaesthetic.

We have demonstrated that a specific inspiratory initiating reflex exists in animals[4] and that this is suppressed by general anaesthetics.[5] However the region of GV26 is very pain sensitive (a sharp blow to this region being a classic attack in the martial arts) and the present experiment was designed to see if stimulation of GV26 was a more potent initiator of respiration than a more general painful stimulus. Because of its subjective nature it is difficult to quantify pain, but we would suggest that the tibial nerve transfixed with a needle which is moved with a twirling pecking action in the recommended acupuncture fashion[3] provided a powerful arousing stimulus.

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TABLE I: LATENCY BETWEEN RESPIRATORY ARREST AND THE FIRST INSPIRATION (AFTER A FIXED DELAY BEFORE NEEDLING STARTED) IN FIVE SHEEP NEEDLED AT GV26; THE TIBIAL NERVE; NOT NEEDLED (CONTROL).

Experiment	GV26	Tib	Control
1. 60s delay	6	46	95
before needling	6	89	89
	12	97	82
2. 60s delay	84	17	64
	36	56	54
	18	34	26
3. 30s delay	14	22	35
	20	18	34
	16	37	30
4. 60s delay	6	40	60
	12	68	70
	0	6	14
5. 10s delay	3	7	22
	0	2	18
	5	15	14
Mean ±S.E.M.	$15.9 \pm 5.4_{(5)}$	$36.9 \pm 7.6_{(5)}$	$47.1 \pm 7.2_{(5)}$

Even if acupuncture of GV26 does not exert its effect simply as a powerful arousing stimulus its central action may be associated with neural mechanisms involved in the perception of pain. Naloxone partially antagonises the analgesic effects of acupuncture in man[6] and animals,[7] and is known to displace narcotics from the opiate receptors in the brain.[8] It may be that the opiate receptor system is modulated by acupuncture of GV26. Acupuncture has been reported as useful in treatment of narcotic addiction,[9] and although the clinical nature of this treatment excluded a rigorous analysis of the physiology involved, it at least suggests involvement of endorphin systems.

On the other hand, the time taken to initiate breathing in our experiments was very short, which leads us to suggest a direct neural mechanism is involved in the significant respiratory initiating effect of stimulating acupuncture of point GV26.

This study indicates that acupuncture of point GV26 may provide a method of reversing respiratory arrest in clinical situations.

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BOOK REVIEW

CANINE NEPHROLOGY

by Kenneth C. Bovee
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ISBN 0-932036-12-0

This is a specialised book which the author has written with the aim of keeping students, interns and residents up to date in the developing area of canine nephrology. It provides a summary of canine nephrology from a review of normal renal physiology to the in depth discussion of the pathophysiology and treatment of kidney diseases of the dog. There are 818 pages and 32 chapters, each one covering a different aspect of kidney function, disease or treatment. There are 21 contributors with the author contributing 12 of the chapters.

The dog has been used for studies on many aspects of renal physiology, pathophysiology, immunology and clinical investigation and this book provides the reader with an easily read and understood summary of this work. An understanding of the pathophysiology of disease of any organ system is essential if rational accurate treatment is to be instituted. Therefore this book also provides the veterinary practitioner with explanations as to why certain treatment regimens are indicated for particular renal diseases. There are excellent chapters on acid-base balance, urinary tract obstruction, immunologic injury to the kidney and the management of acute and chronic renal failure.

The knowledge is specialised; therefore the book is likely to have a limited audience. Apart from educators, students, interns and residents for whom this book was primarily produced, it will in addition be of benefit to veterinary practitioners who have a particular interest in renal diseases or who require a more basic and/or detailed understanding of kidney function and disease.

Publication 36.

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Influence of lung stretch receptors on the cough reflex.

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Influence of Lung Stretch Receptors on the Cough Reflex in Rabbits

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Key Words. Cough · Breuer-Hering reflex · Irritant reflexes

Abstract. The cough reflex in anaesthetized rabbits was elicited by irritation of the larynx with ammonia, inhalation of ammonia into the lungs, and mechanical stimulation of the tracheobronchial tree with a catheter. After block of lung stretch receptors with sulphur dioxide and virtual abolition of the Breuer-Hering inflation reflex, the cough response to all three stimuli was far less frequent and, when it occurred, was weaker especially with respect to the expiratory efforts. It is concluded that the Breuer-Hering reflex enhances the cough reflex, although modification of the sensitivity of tracheobronchial cough and irritant receptors by sulphur dioxide may also influence the cough.

Introduction

Both clinical observations and experimental research show that there are changes in respiratory reflex activity during diseases of the respiratory system [3, 13, 15, 16, 20, 21]. In particular, there are qualitative and quantitative changes in one of the most important and powerful of the respiratory reflexes, namely the cough reflex [13]. *Korpáš* et al. [11, 12, 14] found that, during slight experimental inflammation of the airways, the cough reflex induced by mechanical stimulation of the mucous membrane increases in intensity, whereas the intensity decreases below control values when the inflammatory changes are very intensive. In addition, coughing shows special features during the onset of various types of experimental airways' inflammation [6, 8, 9]. There may be an increase in cough intensity followed by a decrease in sensitivity until the intensity returns to control values for healthy animals. This is an important observation because a large increase in cough intensity can be harmful [13, 19], whereas its depression or absence may be even more dangerous in certain situations [15]. Extreme changes in the strength of the reflex could play a part in the pathogenesis of respiratory tract diseases.

[1] We are grateful to the Wellcome Trust for financial support for J.H.

The primary changes in the strength of the cough reflex probably originate close to the site of inflammation where the receptors for the cough reflex are localised. It is generally accepted that the receptors of the cough reflex belong to the class of rapidly adapting 'irritant receptors' within the walls of the larger airways [22]; however, there have been few studies of the ways their properties may be changed in inflammatory conditions. Nor is it known to what extent other types of respiratory tract receptors may contribute to the overall response.

In the present paper we try to answer the question whether lung stretch receptors influence the cough reflex. *Hanáček and Korpáš* [7] have shown that lung inflations that stimulate lung stretch receptors enhance the expiration reflex from the larynx of rabbits. *Bucher* [4] has suggested that stimulation of these receptors can strengthen the cough, and conversely, that block of receptors reduces the intensity of cough. We have taken advantage of the observation that lung stretch receptors can be blocked selectively and transiently by sulphur dioxide (SO_2) in the rabbit [5]. An abstract of some of the results has been published [10].

Methods

New Zealand white rabbits (2.9–4.5 kg) were anaesthetized with sodium pentobarbitone (Sagital, 30 mg/kg i.v.). Catheters were tied into the right femoral artery and vein and the trachea was cannulated. Supplementary doses of pentobarbitone were given as necessary to maintain surgical anaesthesia. Air flow was measured by a Fleisch pneumotachograph connected to the tracheal cannula. Tidal volume was obtained by integrating the air flow signal electrically. Transpulmonary pressure was measured by a differential capacitance manometer connected between a catheter in the lower right intercostal space and the trachea. A second tracheal cannula, directed cranially, was inserted for administration of NH_3 to the larynx. An opening was made in the ventral pharyngeal wall to limit the entry of NH_3 into the mouth and nose.

Sulphur dioxide mixtures were made up in a polyethylene Douglas bag, by mixing air and pure SO_2 (BOC Special Gases) via two rotameters. The concentration of SO_2 was measured using a Dragor Normalar gas sampling system. The SO_2 mixture (200 ppm) was drawn across the rabbit's trachea by suction pump via a T-piece attached to the tracheal cannula. The pump was adjusted so that pressure at the tracheal cannula was atmospheric, the pressure in the Douglas bag being slightly positive. The animals breathed the SO_2 mixture for 10 min or longer to abolish lung stretch receptor activity and the Breuer-Hering reflex [5].

We elicited the cough reflex by administration of 2 ml of 1×10^{-3} NH_3 (molecular concentrations) to an airstream (0.5 litre/min) blown through the cranial tracheal cannula and the larynx (laryngeal cough). From the tracheobronchial tree we elicited the cough by mechanical stimulation 5 times during 5 s with a soft polyethylene catheter or by administering 1.0 or 2.0 ml of 1×10^{-3} or 2×10^{-3} NH_3 (and rarely 5 or 10×10^{-3} NH_3) to the inspired air. To test activity of pulmonary stretch receptors we usually used the Breuer-Hering reflex, by inflating the lungs to a maintained pressure of 1 kPa. If the inflation caused no prolongation of expiration we concluded that stretch receptor activity had been blocked.

For evaluation of the cough reflex, we measured the number of cough efforts (NE), the frequency of cough efforts (f), the intensity of the maximal cough effort (IME) and the intensity of cough attack (IA, the sum of all the amplitudes of cough efforts in one cough attack). Inspiratory and expiratory phases were assessed separately, and intensities were assessed from intrapleural pressures.

Results

Cough Elicited from the Larynx by NH_3

Administration of NH_3 into the larynx induced cough in each of 21 times in 10 rabbits. The measured changes are shown

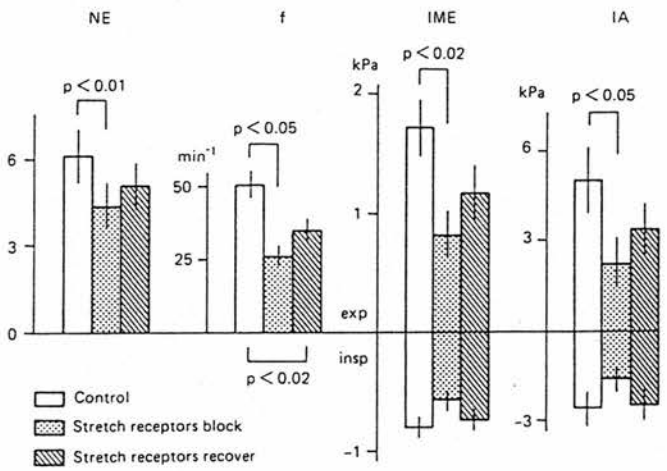

Fig. 1. Quantitative parameters of cough in response to stimulation of the larynx with ammonia. NE = Sum of the single cough efforts during one cough attack; f = number of cough efforts per minute; IME = intensity of maximum cough effort; IA = intensity of cough attack, i.e. sum of all amplitudes of the cough efforts during one cough attack. For IME and IA, values above the line are expiratory (exp), below the line are inspiratory (insp). Values are given for controls, during stretch receptor block and during recovery.

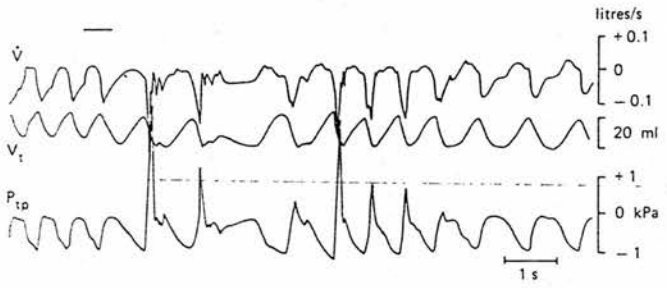

Fig. 2. Record of cough in response to stimulation of the larynx in a rabbit. From above down, ventilatory airflow ($\dot{V}$), tidal volume (V_t) and transpulmonary pressure (P_{tp}). The black bar at the top indicates the period of NH_3 application to the larynx.

in figure 1, and a record of a cough in figure 2. The Breuer-Hering inflation reflex in these animals was initially strong; lung inflation with a pressure of 1.0 kPa extended the expiratory time by a mean of 22-fold.

Immediately after the lung stretch receptors had been blocked with SO_2, inflation of the lungs only extended expiratory time by 1.4-fold, indicating that the Breuer-Hering reflex and pulmonary stretch receptor activity had been virtually abolished. On stimulation of the larynx with NH_3, cough was absent in 6 tests out of 19. For the 13 positive responses, there were statistically significant reductions of about 40–55% in all the measured variables of cough, except the intensity of inspiratory efforts (fig. 2).

At 20–30 min after the end of SO_2 administration, the Breuer-Hering reflex had about half recovered (expiratory time prolongation, 12-fold). Cough was induced in 15 tests out of 20, and the parameters of cough were about half restored to control values (fig. 1).

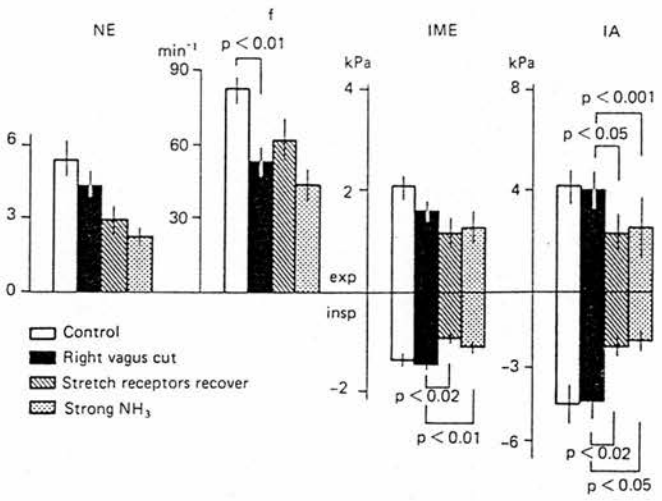

Fig. 3. Quantitative parameters of cough in response to inhalation of ammonia into the lungs. Presentation as for figure 1. Values are given for controls, for rabbits with right vagus cut, during recovery from stretch receptor block and in response to stronger ammonia during recovery. The values for cough during stretch receptor block are omitted because only 1 cough occurred in 13 tests.

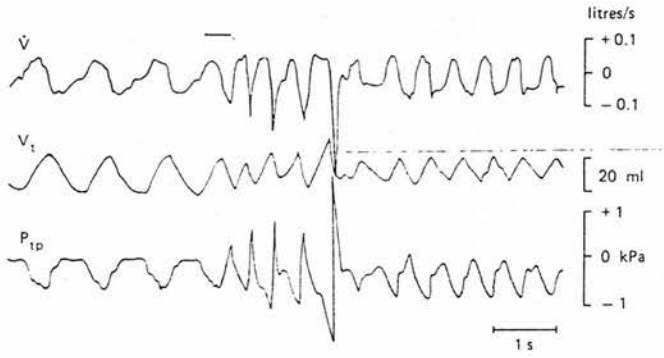

Fig. 4. Record of cough in response to introduction of ammonia into the lungs. Presentation as in figure 2.

Cough Elicited from the Tracheobronchial Tree by NH_3

We stimulated the tracheobronchial mucosa by administering NH_3 in a single inspiration. This caused an immediate cough in each of 28 tests in 18 rabbits, which could be elicited repeatedly at 10- to 15-min intervals. Quantitative values are shown in figure 3, with a representative record in figure 4. The Breuer-Hering inflation reflex initially prolonged expiratory time an average of 30-fold. For 8 rabbits with both vagi intact, the lung stretch receptors were blocked with SO_2, the Breuer-Hering reflex now causing expiratory prolongations of only 1.5-fold. The cough reflex was induced only once out of 13 tests with NH_3, a response omitted from figure 3.

For a second group of 10 rabbits, we tested the effect of right vagotomy on NH_3-induced cough. NH_3 still induced

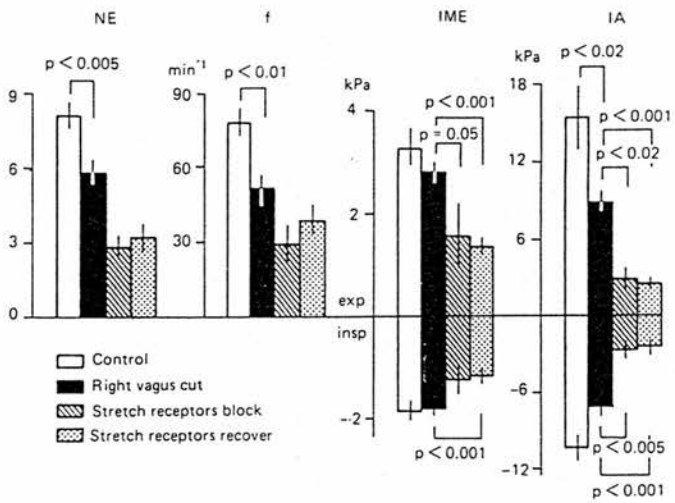

Fig. 5. Quantitative parameters of cough in response to mechanical stimulation of the tracheobronchial tree. Presentation as for figure 1. Values are given for controls, for rabbits with right vagus cut, during stretch receptor block and during recovery.

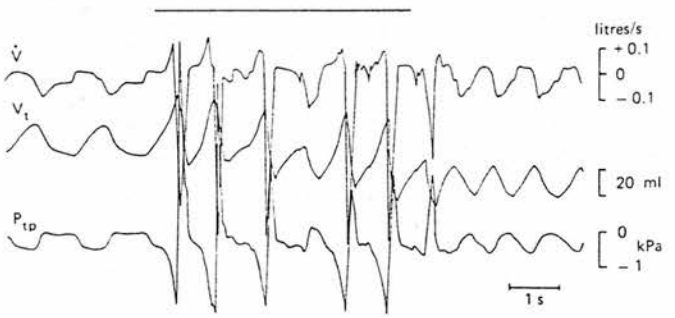

Fig. 6. Record of cough in response to mechanical stimulation of the tracheobronchial tree. Presentation as in figure 2.

cough in 16 of 17 tests, although the frequency of cough efforts was reduced (fig. 3). Unilateral vagotomy weakened the Breuer-Hering reflex, expiratory time being prolonged only 8.5-fold. After block of the Breuer-Hering reflex by SO_2 in the right vagotomized rabbits, NH_3 only caused coughing in one of 13 tests (not included in fig. 3).

For both groups of rabbits, 30 min after stretch receptor block, the Breuer-Hering reflex prolonged expiration 9.8-fold, and NH_3 stimulated coughing in 13 of 28 tests in 12 of the rabbits. Compared with controls before stretch receptor block, there was a significant decrease in inspiratory values of IME and in inspiratory and expiratory values of IA (fig. 3). In the 6 rabbits which did not cough with NH_3 we tried a stronger stimulus (2 ml of 5 or 10×10^{-3} NH_3). Cough occurred in 11 of 13 tests, with measured

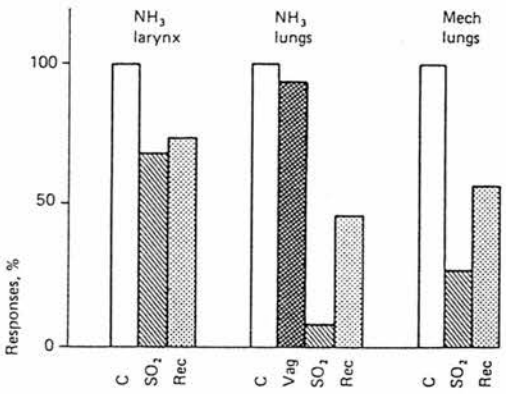

Fig. 7. Occurrence of cough efforts after NH_3 in the larynx, NH_3 in the lungs and mechanical (mech) stimulation of the tracheobronchial tree. Ordinate, percent of positive responses to tests. C, controls; SO_2, during stretch receptor block by sulphur dioxide; Rec, during recovery from block; Vag, rabbits with right vagus cut but without stretch receptor block.

values similar to those of effective weaker stimuli (fig. 3).

Cough Induced from the Tracheobronchial Tree by a Catheter

Mechanical stimulation of the tracheobronchial tree always caused coughing (n = 28, fig. 5, 6). In 9 rabbits out of 12, the right vagus nerve was cut. This did not reduce the animals' ability to cough after a mechanical stimulus (23 out of 23 tests). However, the parameters of this type of cough were significantly decreased (fig. 5). The intensity of the Breuer-Hering reflex was similar to the other control groups. During stretch receptor block, the cough reflex appeared in only 4 of 15 stimulations, and the intensity of those coughs that occurred was weaker than for the controls (fig. 5). After recovery from stretch receptor block, mechanical stimulation of the tracheobronchial tree caused cough in 12 of 21 tests, with significantly lower expiratory and inspiratory values of IME and IA (fig. 5).

Discussion

Bucher [4] discussed the possible role of lung stretch receptors in the cough reflex. He suggested that, during the inspiratory effort which is an integral part of the cough reflex, the stretch receptors are more intensively stimulated, thereby increasing the inhibitory influence on central inspiratory activity and thus strengthening the subsequent expiration.

Bishop and Bachofen [2] showed that positive pressure breathing excites expiratory activity in the abdominal muscles, via lung stretch receptors. *Palaček and Chválová* [17], found that the expiratory flow rate in rats increases when tidal volume is longer by an effect partly dependent on vagal activity. Thus, inspiratory volume influences the following expiratory effort. In addition, *Bucher* [4] claimed that the intensity of the cough reflex could be reduced by anaesthetizing lung stretch receptors. Our results confirm the fact that inhalation of SO_2 in rabbits almost completely blocks the Breuer-Hering reflex [5]; they are consistent with a decrease in cough intensity due mainly to lung stretch receptor block. *Hanáček and Korpáš* [7] showed that the expiration reflex from the larynx of rabbits was stronger when the lungs were inflated, and concluded that this was due to the Breuer-Hering reflex.

We have provoked cough in three different ways with very different patterns of response (fig. 2, 4, 6). In all three instances

the cough was inhibited by SO_2 inhaled into the lungs, and figure 7 summarizes the *occurrence* of cough (percentages of tests) with each group of experiments. Unilateral vagotomy had no such effect. When the cough did occur during lung stretch receptor block, with each type of cough the expiratory efforts were weaker.

The fact that cough due to *laryngeal* stimulation was prevented or inhibited by SO_2 inhaled *into the lungs* with block of pulmonary stretch receptors shows that the activity in these receptors sensitizes or enhances the cough reflex. However SO_2 in the lungs may also have a depressant action on cough receptors there, since the Breuer-Hering reflex recovered more quickly than did the intensity of cough. The total effect of a cough stimulus must depend not only on the degree of stimulation of cough receptors, but also on the effect of the cough stimulus on other receptors, and on the interactions due to the involvement of secondary reflex stimulations.

Cough intensity changes, due to stimulation of the tracheobronchial tree by NH_3 with block of stretch receptor activity, are complicated by the fact that two irritant chemicals were used, SO_2 to block stretch receptors and NH_3 to stimulate cough and irritant receptors. Right cervical vagotomy, presumably halving the number of active cough receptors, did not significantly decrease the parameters of cough, except the frequency of cough efforts. However, block of stretch receptors by SO_2 significantly limited the animals' ability to cough in response to stimulation of the airways by NH_3, and reduced the intensity of those coughs that occurred. The question arises whether the decrease in cough was due to the lack in activity of stretch receptors or by a changed sensitivity of cough receptors. A tentative answer is provided by our results. Figure 3 shows that the cough intensity/inspiratory values of IME and both inspiratory and expiratory values of IA remained depressed even when the stretch receptors had partly recovered their function. Thus, it is possible that the behaviour of cough or irritant receptors was changed by SO_2 in our experiments; although stronger concentrations of ammonia still provoked coughing, it was at reduced intensity.

Figure 1 shows that, for cough from the larynx, expiratory values of IME and IA can be significantly decreased by block of the Breuer-Hering reflex, while inspiratory values are not significantly changed. Figure 3 shows that, for cough from the lungs, block of the Breuer-Hering reflex has a greater effect on expiratory than on inspiratory responses. Thus stretch receptors have a more powerful influence on the strength of expiration. This conclusion agrees with that of *Bishop* [1] who showed that the inhibitory effect on inspiratory muscles of the Breuer-Hering inflation reflex does not match in threshold the onset of tonic activity in the expiratory muscles.

Mechanical and chemical stimulations of the airways increase mucus secretion in the airways [18]. It is possible that a decrease in cough receptor sensitivity could be due by a thicker mucus layer, that would hinder penetration of NH_3 to the receptors. However, this possibility would not explain the results with mechanical stimuli which would easily penetrate the thickened mucus layer and so stimulate cough receptors, or those with a stimulus to the larynx.

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EFFECT OF CHANGES IN AIRWAY PRESSURE ON BREATHING PATTERN IN CONSCIOUS DOGS

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SUMMARY

The effect of brief (400 ms) or sustained (several breaths) application of positive or negative intrapulmonary air pressure was studied in three exercising conscious dogs. Changes in inspiratory duration (T_I), expiratory duration (T_E) and tidal volume (V_T) were measured with the dogs' vagus nerves at body temperature and cooled to a temperature which abolished the Hering–Breuer inflation reflex (7 °C). Comparison of these results with those obtained with anaesthetized dogs leads us to suggest that there exists in conscious dogs a reflex inspiratory promoting drive.

INTRODUCTION

It is generally accepted that the slowly adapting pulmonary stretch receptors described by Adrian (1933) have an important role in the breath by breath regulation of breathing. They appear to be responsible for the prolongation of expiration in response to lung inflation described by Breuer (1868) and are involved in the increased frequency of respiration with deflation of the lungs described in the same paper. More recently termination of inspiration has been attributed to their activity (Clark & von Euler, 1972). This activity is modulated by changes in lung volume, and reflexes provoked by forced inflation and deflation of the lungs provide an index of the activity of the stretch receptor system. General anaesthetics modify both the central sensitivity to this vagally mediated afferent information (Severinghaus & Larson, 1965) and the activity of the receptors themselves (Coleridge, Coleridge, Luck & Norman, 1964). We have demonstrated (Davies & Roumy, 1978) that rapid and brief inflation or deflation of the lungs of anaesthetized rabbits provokes an inspiratory augmenting vagal reflex, probably associated with the activity of rapid adapting receptors. The effects of this activity differed depending on the phase of breathing in which it was triggered.

The object of the present study was to investigate if an inspiratory augmenting reflex could be demonstrated in conscious dogs, to determine the effect of general anaesthesia on this and the reflexes produced by sustained lung inflation and deflation, and to estimate the part played by slowly adapting pulmonary receptors in these reflexes.

To this end we compared these reflexes in groups of conscious and anaesthetized dogs. We selectively blocked conduction in the vagus nerves to investigate the contribution made to these reflexes by slowly adapting pulmonary stretch receptors and concluded that general anaesthesia suppresses vagally mediated inspiratory augmenting activity that probably arises from rapidly adapting receptors in the lung.

METHODS

Three dogs of mixed breed (weight 22–26 kg) were trained to exercise on a treadmill. Once the dogs were trained, both cervical vagosympathetic nerve trunks were exteriorized surgically, and a 3–4 cm length of each nerve was implanted in a tube of cervical skin. This operation created permanent cervical vagal loops that separated the enclosed nerves from other cervical structures and thus made them accessible for reversible blockade by cooling in the conscious state. Three weeks later, a second operation was performed on the same animals to create a permanent side-hole tracheostomy. Another 3–4 weeks was allowed for healing, and excerise training was continued during this period.

In unanaesthetized dogs, ventilation is less variable during mild exercise than at rest. This is probably more due to the exercise occupying the dogs attention and thus preventing them sniffing at objects of interest than to any other factor. Therefore, all studies were performed while the dogs walked at 3 miles per hour on a level treadmill and breathed via a cuffed endotracheal tube (10–12 mm i.d.); one end of the tube was inserted into the trachea through the tracheostomy, and the other end of the tube was connected to a Rudolph valve and pneumotachograph. The dead space of the apparatus was 45 ml and the airflow resistance was 2·6 $cmH_2O/l.s$ at a flow rate of 0·5 l/s. The tracheal pressure was monitored through a lateral hole at the end of the endotracheal tube via a catheter attached to a pressure transducer (Validyne, DP7). Inspiratory airflow was measured with a pneumotachograph (Fleisch no. 2) connected to a differential pressure transducer (Validyne model DP45). Tracheal gas was sampled (2 ml/min) and analysed continuously for CO_2 and O_2 using a rapid response (time constant, 0·08 s) mass spectrometer (Perkin-Elmer model MGA 1100). Heart rate was monitored by a three-lead electrocardiograph attached to the chest wall. All analog signals were recorded on a multi-channel recorder (Honeywell Visicorder model 1508C), and simultaneously analysed by an on-line digital computer (Digital Equipment model PDP 11/34). Breath by breath analyses of tidal volume, respiratory frequency, minute volume of ventilation, and of the durations of inspiration and expiration made by the dogs were calculated by the computer. These were displayed continuously on a cathode ray storage oscilloscope (Tektronix model 5115) and printed on paper (Versatec Printer) with statistical analysis. Results analysed by the computer were routinely compared with those obtained from analog tracings of the Visicorder for accuracy. During the experiment we avoided any external disturbance to the dog such as noise or movement and the experimenters were hidden from sight. The dogs needed no prompting to walk on the treadmill and would step into position immediately on entering the laboratory in anticipation of the exercise. To prevent them panting, we clipped the dog's hair and maintained a cool atmosphere in the laboratory (18–19 °C).

Cooling blockade of conduction in the vagus nerves was achieved by circulating cold alcohol through the copper radiators that were fitted around the cervical vagal loops. The temperature of alcohol leaving the radiators, which was the same as the temperature of the inner surface of the radiators, was monitored continuously by a probe (Yellow Springs Instrument no. 401) enclosed in the outlet tube of circulating coolant.

At 200 ms after either the beginning or end of inspiratory air flow, a system of electromagnetic valves connected the endotracheal tube to a 40 gallon drum containing air maintained at ± 20 cmH_2O relative to atmospheric pressure for pressure pulses and ± 10 cmH_2O for steps. Pulses of pressure were of 400 ms duration. Steps of positive pressure were maintained for three inspiratory efforts and steps of negative pressure for about eight breaths. The pressure system was flushed with room air to prevent the build up of carbon dioxide. The connecting tubing used was of wide bore but to test the possible effects of any added resistance it might have imposed on the dogs breathing it was connected and disconnected from the endotracheal tube and found not to affect the pattern of breathing.

The conscious dogs were allowed to walk for 4 min to reach a steady state. Steps of positive or negative pressure were alternately applied to their lungs. These were followed by alternating pulses of positive or negative pressure applied in inspiration or expiration. The vagi were then cooled to 7 °C and maintained at that temperature for 10 min and while the steps and pulses were repeated. In two dogs the temperature of the vagi was dropped to 0 °C and the steps and pulses repeated. Respiratory pattern began to be affected at a vagal temperature of 15 °C.

Three dogs (weight 20–30 kg) were anaesthetized with choloralose and urethane (50 and 500 mg/kg respectively) and subjected to the steps and pulses of pressure via an endotracheal tube. These dogs were then vagotomized and the steps and pulses repeated. It was necessary to reduce the pressure of the inflation steps in these dogs (vagi intact) to 5 cmH_2O as the apnoea produced by an inflation of 10 cmH_2O was excessively long. Inspiratory duration (T_I), expiratory duration (T_E) and tidal

volume (V_T) are expressed relative to the preceding control breath, and thus values above 1 indicate increase and below 1 decrease.

Significance of differences between control and test breaths in an individual dog determined by Student's t test.

RESULTS

The mild exercise of walking on the treadmill produced little change in the pattern of breathing. Mean frequency increased from thirteen to eighteen per minute.

The effect of lowering the temperature of the radiators surrounding the vagi on the pattern of breathing of three conscious dogs is shown in Table 1. Changes in breathing pattern began to take place at approximately 15 °C.

In these dogs a sustained airway pressure of + 10 cmH_2O relative to atmospheric pressure inflated the lungs to approximately twice eupnic V_T. The pooled results from conscious and anaesthetized dogs is shown in Fig. 1. In the conscious dogs the second T_I (with vagi warm) and the first T_I (vagi cool) after inflation were always augmented, with increased T_I and V_T.

Fig. 2 summarizes the effect of sustained deflation to − 10 cmH_2O relative to atmospheric pressure on the pattern of breathing of conscious and anaesthetized dogs. In conscious dogs when deflating pressure was released there was an augmentation of inspiration. With vagi warm this occurred in the first or second breath in four out of nine trials. With vagi cool augmentation occurred in the first breath after every release of deflating pressure. No augmentation was seen with the anaesthetized dogs.

Positive pressure pulses in inspiration reduced the duration of that inspiration in the conscious dogs with vagi warm or cooled and in the anaesthetized dogs (Table 2). Negative pressure pulses prolonged inspiration (Table 2).

Pulses of positive pressure applied during expiration produced a transient increase in lung volume of less than 20% eupnic V_T and the effects are summarized in Fig. 3. In conscious dogs with vagi cool there was little change in the expiration containing the pulse but the following T_I was shortened. Pulses of negative pressure in expiration produced the effects summarized in Fig. 4. With vagi warm, T_E was not significantly changed in sixteen out of twenty trials. In the remaining four, inspiration was immediately initiated by the pulse. With vagi cool, inspiration was immediately initiated in fifteen out of eighteen trials. In the anaesthetized dogs T_E was consistently shortened to the value shown in Fig. 4.

Cooling the vagi to 0 °C abolished all responses to steps or pulses of pressure in the two dogs tested.

DISCUSSION

The suggestion that mechanisms involved in the Hering–Breuer inflation reflex terminate inspiration, thereby limiting tidal volume has been questioned by Fishman, Phillipson & Nadal, (1973). These authors proposed that the inhibition of inspiration generated by lung inflation delays the onset of the subsequent inspiration which is terminated by the activity of rapidly adapting receptors. We have used their method of cooling the vagus nerves of conscious dogs to investigate the effects of sustained and brief inflations and deflations of the lungs on the pattern of breathing. We realize that the degree of differential vagal blockade we could achieve was limited and have therefore assessed the degree of blockade in terms of reflex responses, in particular the inhibition of ventilation by maintained lung inflation. It is unlikely that transmission of impulses from pulmonary stretch receptors

Table 1. *Effect of vagal cooling to 7 °C on the pattern of breathing of three conscious dogs*

Radiator temperature	Dog 1			Dog 2			Dog 3		
	T_I	T_E	V_T	T_I	T_E	V_T	T_I	T_E	V_T
18 °C	1·23 ± 0·11 s	1·9 ± 0·1 s	440 ± 90 ml	1·1 ± 0·04 s	2·4 ± 0·11 s	560 ± 30 ml	0·9 ± 0·09 s	2·6 ± 0·08 s	660 ± 120 ml
7 °C	1·7 ± 0·04 s	1·3 ± 0·04 s	470 ± 20 ml	1·3 ± 0·08 s	1·5 ± 0·03 s	530 ± 30 ml	0·7 ± 0·08 s	1·3 ± 0·17 s	450 ± 40 ml

n = 15 breaths, mean ± S.E.M.

Table 2. *Effect of positive or negative pulses of pressure (20 ml H_2O) applied in inspiration to the lungs of conscious and anaesthetized dogs (mean ± S.E.M.)*

	Negative pulse								
	Breath before			Pulse breath			Breath after		
	T_I (s)	T_E (s)	V_T (ml)	T_I	T_E	V_T	T_I	T_E	V_T
Conscious, vagi warm (n = 17)	1·2 ± 0·07	2·3 ± 0·1	580 ± 45	1·5 ± 0·09	2·1 ± 0·17	500 ± 55	1·1 ± 0·08	2·1 ± 0·12	500 ± 38
Conscious, vagi cool (n = 17)	1·3 ± 0·12	1·1 ± 0·04	470 ± 23	1·5 ± 0·12	1·1 ± 0·04	520 ± 33	1·4 ± 0·13	1·1 ± 0·06	511 ± 50
Anaesthetized (n = 17)	0·8 ± 0·06	2·8 ± 0·09	300 ± 50	1·1 ± 0·04	2·9 ± 0·18	406 ± 65	0·83 ± 0·05	2·8 ± 0·11	300 ± 54
	Positive Pulse								
	Breath before			Pulse breath			Breath after		
	T(s)	T_E	V_T (ml)	T_I	T_E	V_T	T_I	T_E	V_T
Conscious, vagi warm (n = 12)	1·2 ± 0·08	2·1 ± 0·09	508 ± 30	1·1 ± 0·06	2·3 ± 0·07	841 ± 50	1·3 ± 0·06	2·0 ± 0·1	587 ± 70
Conscious, vagi cool (n = 12)	1·3 ± 0·12	1·1 ± 0·04	437 ± 30	0·9 ± 0·04	0·6 ± 0·11	672 ± 30	0·5 ± 0·06	1·1 ± 0·04	256 ± 47
Anaesthetized (n = 12)	0·9 ± 0·05	2·6 ± 0·06	300 ± 50	0·7 ± 0·04	2·3 ± 0·07	300 ± 80	0·8 ± 0·05	2·5 ± 0·1	300 ± 50

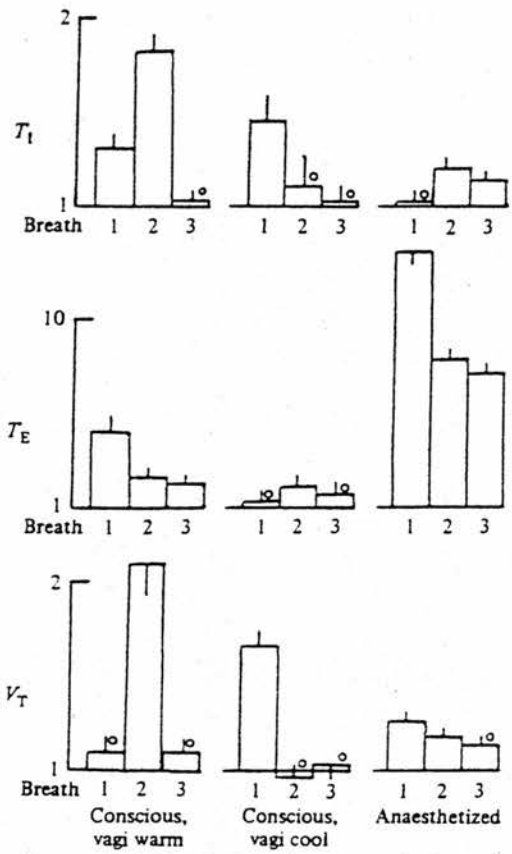

Fig. 1. Positive pressure step. Changes in T_I, T_E and V_T relative to control values (taken as 1) for successive breaths after a sustained positive pressure of 10 cmH_2O was applied to the airways during the inspiration of breath 1. Augmentation of inspiration and tidal volume is transferred from breath 2 to breath 1 by cooling the vagi. No clear augmentation of inspiration is seen when the animal was anaesthetized. (Bars = S.E.M.; 0 = not significantly different from control, $P < 0{\cdot}05$.) Absolute values of control breaths: $T_I = 0{\cdot}91 \pm 0{\cdot}09$, $T_E = 2{\cdot}12 \pm 0{\cdot}2$, $V_T = 555 \pm 43$, conscious, vagi warm, $n = 9$. $T_I = 1{\cdot}2 \pm 0{\cdot}21$, $T_E = 1{\cdot}12 \pm 0{\cdot}2$, $V_T = 458 \pm 50$, conscious, vagi cool, $n = 6$. $T_I = 0{\cdot}6 \pm 0{\cdot}03$, $T_E = 3{\cdot}01 \pm 0{\cdot}09$, $V_T = 350 \pm 45$, anaesthetized, $n = 7$.

(p.s.r.) was completely blocked by cooling. Paintal (1966) has shown that high frequency activity in a nerve is blocked at higher temperatures than low frequency activity. We have therefore based our conclusions on the premise that there is, as yet, no evidence for opposing actions of pulmonary stretch receptor activity blocked at different temperatures.

We have expressed the changes in duration of the phases of breathing in our experiments relative to a preceding control breath. It should be remembered that the patterns of breathing under control conditions in the vagi warm, vagi cool, and anaesthetized states were different, but these differences and the frequency accelerating effect of the mild exercise were not so extreme as to obliterate the changes produced by changes in intrapulmonary pressure.

We inflated and deflated the dogs' lungs with and without differential vagal block and analysed the changes in duration of T_I and T_E. With vagi intact sustained lung inflation produced the classical Hering–Breuer apnoea. However T_I of the first breath during lung inflation was increased to a value greater than that before inflation. This prolongation, accompanied by an increase in V_T, was made against increased pulmonary stretch receptor

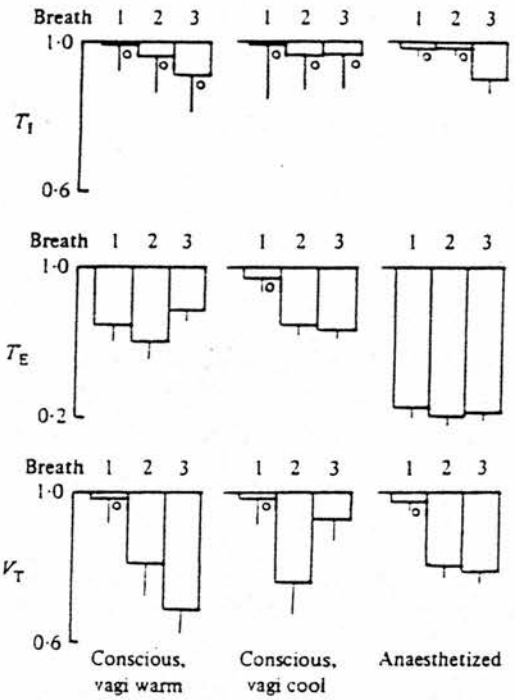

Fig. 2. Negative pressure step. Changes in T_I, T_E and V_T relative to control values (taken as 1) for successive breaths after a sustained negative pressure of 10 cmH_2O was applied to the airways during the expiration of breath 1. (Bars = S.E.M.; 0 = not significantly different from control, $P < 0{\cdot}05$.) Absolute value of controls: $T_I = 1{\cdot}05 \pm 0{\cdot}1$ s, $T_E = 2{\cdot}29 \pm 0{\cdot}2$ s, $V_T = 415 \pm 50$ ml, conscious, vagi warm, $n = 9$. $T_I = 1{\cdot}07 \pm 0{\cdot}1$ s, $T_E = 1{\cdot}23 \pm 0{\cdot}25$ s, $V_T = 465 \pm 52$ ml, conscious, vagi cool, $n = 6$. $T_I = 0{\cdot}62 \pm 0{\cdot}03$ s, $T_E = 2{\cdot}84 \pm 0{\cdot}11$ s, $V_T = 250 \pm 20$ ml, anaesthetized, $n = 7$.

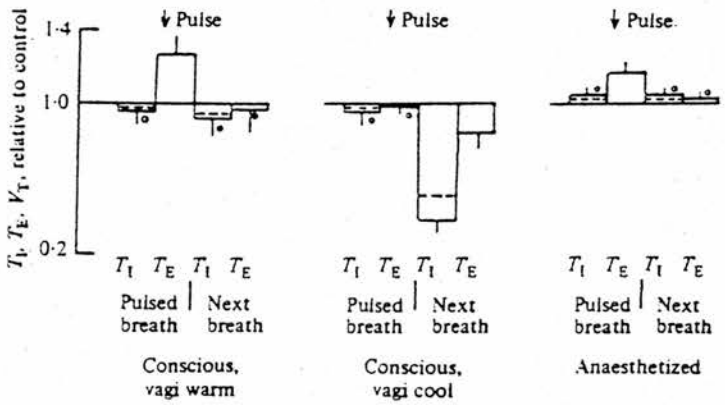

Fig. 3. Positive pressure pulse in expiration. Changes in T_I, T_E and V_T relative to control values (taken as 1) when a 400 ms pulse of +20 cmH_2O was applied to the airways during expiration. V_T is represented by the broken line in inspiration. Note, when the vagi were cool the T_E extending effect of the pulse was abolished and the duration of the next T_I was reduced. (Bars = S.E.M.; 0 = not significantly different from control, $P < 0{\cdot}05$.) Absolute values for controls: $T_I = 1{\cdot}19 \pm 0{\cdot}08$ s, $T_E = 1{\cdot}99 \pm 0{\cdot}18$ s, $V_T = 486 \pm 40$ ml, conscious, vagi warm, $n = 19$. $T_I = 1{\cdot}49 \pm 0{\cdot}13$ s, $T_E = 1{\cdot}17 \pm 0{\cdot}04$ s, $V_T = 475 \pm 46$ ml, conscious, vagi cool, $n = 18$. $T_I = 0{\cdot}93 \pm 0{\cdot}01$ s, $T_E = 2{\cdot}47 \pm 0{\cdot}05$ s, $V_T = 300 \pm 30$ ml, anaesthetized, $n = 8$.

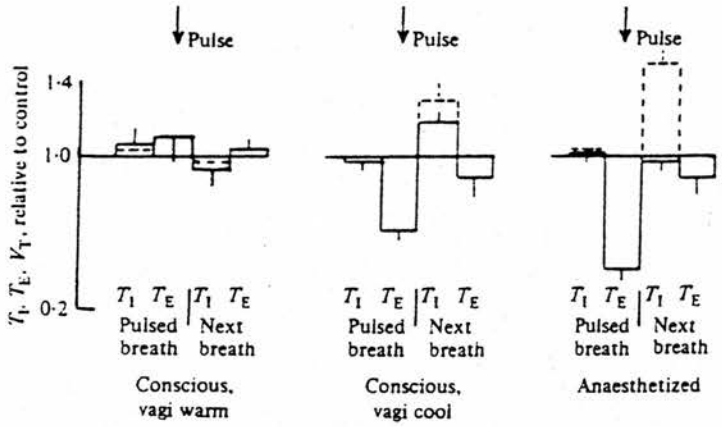

Fig. 4. Negative pressure pulses in expiration. Changes in T_I, T_E and V_T relative to control values (taken as 1) when a 400 ms pulse of -20 cmH_2O was applied to the airways during expiration. V_T is represented by the broken line in inspiration. When the vagi were warm sixteen out of twenty pulses produced a small inspiratory flow (duration, 0·43 s) due to elastic recoil of the lungs and chest wall. Four out of twenty pulses provoked an immediate full inspiration (duration, 1·33 s) coincident with the trailing edge of the pulse. These four have been excluded from this Figure. With the vagi cool fifteen out of eighteen pulses produced immediate full inspirations of the duration indicated in the Figure. The remaining three pulses did not significantly alter the duration of expiration. No immediate inspiration was provoked by pulses in the anaesthetized dogs. Absolute values of controls: $T_I = 1{\cdot}23 \pm 0{\cdot}04$ s, $T_E = 2{\cdot}25 \pm 0{\cdot}13$ s, $V_T = 600 \pm 30$ ml, conscious, vagi warm, $n = 20$. $T_I = 1{\cdot}33 \pm 0{\cdot}12$ s, $T_E = 1{\cdot}29 \pm 0{\cdot}07$ s, $V_T = 450 \pm 60$ ml conscious, vagi cool, $n = 18$. $T_I = 0{\cdot}98 \pm 0{\cdot}16$ s, $T_E = 2{\cdot}72 \pm 0{\cdot}07$ s, $V_T = 312 \pm 10$ ml, anaesthetized, $n = 8$.

activity produced by the lung inflation. Increased stretch receptor activity could not have been effective in terminating T_I under these circumstances. This augmentation of T_I was not abolished by cooling the vagi to the temperature which abolished the apnoea. Under these circumstances (cooled vagi) the augmentation occurred immediately on lung inflation. The singularity of the augmented T_I may be associated with a refractoriness we have seen in rabbits (Davies & Roumy, 1978) and others have seen in cats (Reynolds, 1962). If an augmented breath is triggered in these species a further augmentation of T_I cannot be produced for some time. Augmentation of T_I did not occur in the anaesthetized dogs and this may have been associated with the increased inflation apnoea. Sustained lung deflation produced an insignificant reduction in T_I and a significant reduction in T_E which was not abolished by cooling and was potentiated by anaesthesia. Comparison of the changes in T_I and T_E in response to sustained lung inflation or deflation illustrates the difference in sensitivity of the mechanisms controlling these variables to vagal afferent activity. Augmented inspirations were never seen in the anaesthetized dogs and the T_E shortening effects of deflation were potentiated, which suggests different effects of anaesthesia on the mechanisms initiating and augmenting inspiration.

To restrict stimulation to inspiration or expiration we used brief pulses of positive or negative pressure. Both have been seen to stimulate rapidly adapting receptors, presumably by rapid changes in lung volume (Mills, Sellick & Widdicombe, 1970). In general, inflation increases and deflation decreases activity of pulmonary stretch receptors. We have found by direct recording in a separate series of experiments (A. Davies and M. Roumy, unpublished observation), that rapid inflation of the lungs during inspiration and deflation during expiration is the most potent stimulus to rapidly adapting receptors in rabbits. The nature of the preparation used in the present experiments excluded direct recording but the relative strength of the responses suggested this was also true in conscious dogs.

Pulses of positive pressure were most effective in shortening T_I when the vagi were cool. The shortened T_I was followed by a shortened T_E (Table 2). Negative pulses produced a lengthening of T_I. This lengthening ceased to be significant when the vagi were cooled. The shortening and lengthening of T_I, with positive and negative pressure respectively, could be explained by an inspiratory terminating action of pulmonary stretch receptors. The potentiation of the effect of positive pressure by cooling and anaesthesia may be due to early triggering of the T_I limiting mechanism postulated by Fishman *et al.* (1973).

If termination of inspiration in conscious dogs was brought about by negative feed-back from slowly adapting lung volume receptors it was also clearly influenced by other factors. Increased pulmonary stretch receptor activity could not explain the potentiation of the T_I terminating effect of a brief pulse of positive pressure by vagal cooling. Nor could pulmonary stretch receptor activity explain the unchanged or even increased T_I during sustained inflation which persisted after vagal cooling. Even under anaesthesia, which potentiated the T_E extending effect of lung inflation, T_I was not shortened by inflation. Sustained lung deflation likewise produced large changes in T_E without changing T_I. Exceptions to this stability of T_I were the augmented breaths which occurred on lung inflation by positive pressure or release of lung deflation. In such breaths T_I was increased to 1·7 times the control value and V_T almost doubled. Such breaths have been attributed to increased rapidly adapting receptor activity. If this is so it seems unlikely that rapidly adapting receptors have a role in terminating inspiration if manoeuvres which increase their activity augment T_I.

The increase in T_E produced by brief or sustained lung inflation was abolished by differential cold block. The shortening of T_E by sustained or brief deflations was not abolished by vagal cooling. This suggests two separate mechanisms were involved, the inflation sensitive mechanism may have been pulmonary stretch receptors and the deflation sensitive mechanism receptors with smaller and non-myelinated vagal fibres. The potentiation of the responses to inflation and deflation by anaesthesia suggests that the central mechanism which determined T_E was sensitized to the dominant vagal influence during that expiration.

Changes in breathing frequency in the conscious or anaesthetized dogs used in our experiments were largely brought about by changes in T_E. The mechanisms governing T_E were more sensitive to afferent vagal influences than those governing T_I. In our dogs T_I terminating mechanisms did not appear to be influenced by the levels of sustained lung inflation used, or influenced by a vagal block.

With vagi warm or cool, T_I could be prematurely terminated by *rapid* brief increases in lung volume, or extended into an augmented breath when a sustained increase in lung volume was applied. The most likely vagal afferents involved in these reflexes are those from rapidly adapting receptors and those associated with non-myelinated vagal fibres. We can only speculate that one or the other of these groups of receptors is involved in each of these diametrically opposite effects on T_I.

The control of T_E on our conscious dogs seemed to result from a balance between T_E extending influences (which responded to sustained or brief lung inflations, and were blocked by a vagal cooling) and T_E terminating influences which could pass a cold block. Pulmonary stretch receptors and rapidly adapting receptors together with non-myelinated fibres are respectively the most likely candidates for these two roles. Anaesthesia potentiated the T_E extending effects of sustained lung inflation. The mechanisms governing T_E were still accessible to T_E shortening influences as demonstrated by the profound effects of brief or sustained lung deflation during anaesthesia.

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Time dependent respiratory effects -.

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Time-dependent respiratory effects of injected phenyldiguanide on vagotomized rabbits

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Intravenous injections of phenyldiguanide (PDG) have been used to provoke respiratory reflexes attributed to the activity of receptors associated with unmyelinated vagal fibres from the gut, heart and lungs. Section of the vagi below the diaphragm and injection of local anaesthetic into the pericardial sac (Anand & Paintal, 1980) have been used to abolish afferent activity from the gut and heart respectively. Stimulation by PDG of nerve endings in the carotid region can be abolished by stripping the carotid bifurcations (Dawes, Mott & Widdicombe, 1951). It would be expected that cervical vagal section would almost totally abolish all respiratory reflexes if they only originated from the sites outlined above. We and other workers have demonstrated this in rabbits (Dawes, Mott & Widdicombe, 1951; Karczewski & Widdicombe, 1969; Guz & Trenchard, 1971; Davies, Dixon, Callanan, Huszczuk, Widdicombe & Wise, 1978). On the other hand Miserocchi, Trippenbach, Mazzarelli, Jasper & Hazucha (1978) found little diminution of the effect of PDG after vagotomy. Our present study was intended to resolve this difference and demonstrate the sites at which PDG acts in rabbits.

We measured inspiratory duration (t_I) and expiratory duration (t_E) in 19 New Zealand White rabbits, anaesthetized with sodium pentobarbitone (40 mg/kg), while 30 μg/kg PDG was given (via an intravenous catheter whose tip lay close to the right atrium) to the intact rabbit; after injecting 1 ml 2% xylocaine into the pericardial sac; immediately after bilateral cervical vagotomy; 15 min after vagotomy and after cutting the glossopharangeal nerves near the base of the skull.

The respiratory reflex after injection of xylocaine, 15 min after vagotomy and after cutting the glossopharangeal nerves, was as pronounced as in the intact state, and consisted of an increase in frequency almost totally due to a reduction in t_E. With injections given up to 3 min after bilateral vagotomy the respiratory response was greatly attenuated and variable. We suggest this question of timing may contribute to the differences seen by different groups of workers.

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TRACHEOBRONCHIAL AND LARYNGEAL RESPONSES TO HYPERCAPNIA, HISTAMINE AND CAPSAICIN IN DOGS

RÉPONSES TRACHEOBRONCHIQUE ET LARYNGÉE A L'HYPERCAPNIE, L'HISTAMINE ET LA CAPSAÏCINE CHEZ LE CHIEN

Y. Jammes *, A. Davies **, J.G. Widdicombe ***

ABSTRACT : In nine greyhound dogs, anaesthetized with chloralose-urethane, total lung resistance, volume of an isolated cervical tracheal segment and resistance of the isolated larynx were simultaneously measured. Three stimuli were tested : 1) inhalation of a CO_2-enriched gas mixture ; 2) histamine injected intravenously or administered by aerosol to stimulate primarily lung irritant receptors ; and 3) intravenous injection of capsaicin to stimulate primarily lung C-fibre receptors. The stimuli were applied in three successive conditions : 1) neurally-intact animals ; 2) denervation of the right lung plus cold block of myelinated fibres in the left cervical vagus nerve ; and 3) further blockade of non-myelinated fibres in this nerve. Histamine and capsaicin increased lung and laryngeal resistances, and reduced tracheal volume, and the responses after denervation are consistent with the drugs acting by lung vagal reflexes. In neurally-intact animals, hypercapnia increased total lung resistance, decreased tracheal volume and lowered laryngeal resistance. After elimination of conduction in all myelinated fibres, CO_2-induced changes in lung resistance and in tracheal volume were still present. However, the dilating effect of hypercapnia on the larynx diminished markedly. Elimination of all vagal pulmonary afferents abolished the residual laryngeal response to hypercapnia, lowered and delayed changes in tracheal volume and greatly reduced the increase in lung resistance. The results indicate that the laryngeal response to hypercapnia depends on vagal integrity, but the tracheobronchial constrictor effect of CO_2 is less affected by vagal denervation.

Bronchi ; hypercapnia ; larynx ; lung reflexes ; trachea ; vagus nerves.

Previous studies have shown that ventilation of animals with carbon dioxide-rich mixtures constricts the airways. This applies both to total lung resistance [10, 12, 13, 19] and to an isolated tracheal segment [16, 19, 22]. However, differences in the pattern of response to CO_2 exist between species : airway constriction is stable in dogs when hypercapnia is maintained [16], but the initial constrictor effect is followed by a marked bronchodilatation in cats [12].

There are contradictory results on the mechanism of the bronchoconstrictor effect of hypercapnia. Most authors found that bilateral cervical vagotomy abolished the response in dogs [13, 19]. This abolition was also found after selective afferent vagotomy or blockade of conduction in nonmyelinated vagal fibres in cats [12] ; the last result strongly suggests that CO_2-induced bronchoconstriction results from activation of vagal afferents. Neurophysiological data support these observations : thus a moderate increase in inspired CO_2 concentration activates pulmonary nonmyelinated vagal afferents in cats [11], but has little action in dogs over the physiological range [7]. On the other hand, other authors suggest that hypercapnia acts centrally and not by way of a reflex from airway and lung receptors [10]. This is complicated by observations that constriction of an isolated tracheal segment by hypercapnia is reduced but not completely suppressed by cervical vagotomy [16].

Inhalation of a CO_2-enriched gas mixture lowers laryngeal resistance in the cat [2, 14] ; in one study [3], pulmonary vagotomy had little effect on this response, whereas in another [14] it was prevented or even reversed, suggesting the involvement of lung reflexes.

The present work was performed to study the role of vagal reflexes during inhalation of a hypercapnic gas mixture on total lung resistance, volume of an isolated tracheal segment and the resistance of the isolated larynx in spontaneously breathing dogs. To test the role of pulmonary nonmyelinated afferent fibres, hypercapnic tests were repeated after denervation of one lung plus blockade of conduction in myelinated fibres in the other vagus nerve. To test the role of all vagal afferent fibres, the tests were then done after complete pulmonary denervation. The responses to stimulation of irritant receptors and of C-fibres were tested in each circumstance by measuring the airway responses to histamine and capsaicin ; these results give information on the relative effects of these receptors on different components of airway calibre.

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METHODS

General experimental procedure

Nine adult greyhound dogs weighing 24.7 ± 4.6 (SD) kg were anaesthetized with a mixture of chloralose (2.5 g·100 ml^{-1}) at 0.5 % (w/v) NaCl solution) and urethane (25 mg·100 ml^{-1}) given intravenously. The initial dose was usually 2-3 ml·kg^{-1}. Anaesthesia was maintained with injections through a femoral venous catheter, frequently checking the anaesthetic level as judged by spontaneous movements, the level of ventilation and breathing frequency.

Measurement of total lung resistance (RL)

A T-shaped plastic cannula with one cross-limb closed by a rubber stopper was tied into the lower cervical trachea as caudal as possible and the dog breathed through this cannula (see fig. 1 for general arrangement of apparatus). Care was taken to avoid damage to the recurrent laryngeal nerves, the main motor supply to the larynx, and to the tracheal blood vessels. Airflow and tidal volume (VT) were measured via a Fleisch pneumotachograph attached to this cannula and to a differential induction manometer with electrical integration (Godart GM0577). A differential electromanometer was connected to a widebore needle inserted into the tracheal cannula and to an air-filled rubber catheter tied into a lower right intercostal space; this allowed measurement of transpulmonary pressure (Ptp). Total lung resistance (RL) was obtained by the subtractor method of MEAD and WHITTENBERGER [18] as modified by NADEL and WIDDICOMBE [19]. Ptp and airflow were displayed on the two axes of an oscilloscope. A voltage proportional to VT was subtracted from the total Ptp signal. The slope of the flow *versus* Ptp loop is proportional to RL.

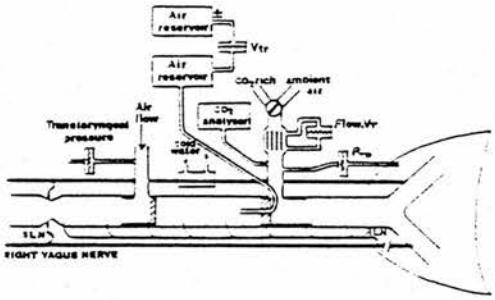

Fig. 1. — Diagram of experimental procedure. SLN : superior laryngeal nerve; RLN : recurrent laryngeal nerve; Ptp : transpulmonary pressure; VT : tidal volume; Vtr : tracheal volume. A copper radiator was used to block conduction in the left vagus nerve and the right nerve was cut in the chest. For further explanation, see Methods.

Changes in tracheal volume (Vtr)

A second similar T-shaped cannula was tied into the trachea between the second and third cartilage. Care was taken to avoid cutting the branches of the recurrent and superior laryngeal nerves and damage to blood vessels. The length and diameter of this isolated tracheal segment were similar in all dogs studied, and the volume was about 20-30 ml. After testing the joints between the trachea and the cannulae for air leaks, volume changes in the segment were measured as previously described [16, 19]. In short, a polyethylene tube was introduced through the lower cannula inside the lumen of the isolated tracheal segment and connected to a 10 l air reservoir. This reservoir and a second identical one were connected to the two sides of a capacitance manometer (operative range ± 1 mmH$_2$O). Both reservoirs could be periodically opened to the atmosphere for equalization of pressures. Occasional drifts of pressure were very slow compared to the rapid changes due to tracheal activity. Contraction of the trachea raised the pressure by a value proportional to the volume change. Calibration was carried out by injecting air from a 1 ml syringe coupled in place of the tracheal cannula to the reservoir system. Absolute changes in tracheal volume (Vtr) were expressed in ml.

Laryngeal resistance (Rlar)

A stream of humidified air was passed through the upper cannula and the larynx at a constant rate of 4 l·min^{-1}, continuously measured by a rotameter. The inflow pressure was measured using a capacitance manometer (Hilger) and represented translaryngeal pressure (Plar); its ratio to flow was taken as laryngeal resistance (Rlar) [22]. The mouth of the dog was opened widely and the tongue and epiglottis were retracted with a large plastic tube in the pharyngeal cavity; thus the air left the upper respiratory tract through the low resistance of the pharynx and the tube, and little passed through the nose. End-tidal CO_2 concentration (F_{CO_2}) was measured with a Beckman Spinco LB1 infrared analyser sampling from the lower tracheal cannula at 300 ml·min^{-1}. This sampling rate was slow compared with tidal tracheal airflow, and tests established that it did not modify measured RL. Blood pressure was recorded with a strain-gauge manometer through a catheter placed in a femoral artery.

During each experiment VT, Ptp, Plar, Vtr changes, F_{CO_2} and blood pressure were recorded on ultraviolet-sensitive paper (Oscillograph UV31, Honeywell) and stored on a 7-channel tape recorder (Ampex SP3000).

Section of nerves and blockade of nervous conduction

After control tests, both vagus nerves were carefully dissected at two levels. The right nerve was cut in the chest below the origin of the recurrent laryngeal nerve. To allow this, the chest was opened and then subsequently closed and spontaneous breathing restored. The result of this section would be that the afferent and efferent supply to the right lung would be interrupted, but the vagal supply to the cervical trachea and the larynx would be intact. The left cervical vagus nerve, which had been freed for about 3–4 cm from the carotid sheath, was placed in a groove on the platform of a copper thermode through which an ice-cold mixture of saline was circulated. The temperature was measured in the outflow stream of the copper thermode.

Two steady-state vagal temperatures were successively achieved. The first, at 5 °C, would block myelinated afferent fibres from the left lung, leaving afferent C-fibres conducting; myelinated motor fibres to the left lung, left cervical trachea and left larynx would also be largely blocked [25]. This is referred to as 'partial vagal block'. The Breuer-Hering reflex was tested by inflating the lungs at 10 cmH$_2$O; its disappearance under cold block at 5 °C indicated blockade of conductance in myelinated fibres. Secondly, lowering the left vagal temperature to 0 °C or cutting the nerve would lead to abolition of all the vagal innervation of both lungs, and the left vagal innervation of the cervical trachea and larynx. The right vagal supply to the latter structures would be intact, as it would with partial vagal block. This condition is referred to as 'complete vagal block'. The terms partial and complete vagal block refer to lung innervation; the cervical trachea and larynx would always retain half or more of their innervation, via the right vagus nerve and both superior laryngeal nerves.

The protocol consisted of a series of control tests first performed in vagus-intact animals: 1) intravenous injection of histamine (20-30 μg·kg^{-1}) via the femoral catheter; 2) inhalation of 1 % histamine acid phosphate aerosol, administered by means of a clinical generator (BOC) with a flow rate of 5 l·min^{-1}; 3) intravenous injection of capsaicin (10-20 μg·kg^{-1}); 4) inhalation of 8 % CO_2 for 30 s, to increase end-tidal CO_2 concentration from 4 to 10-12 %. Changes in RL, Vtr and Rlar were simultaneously measured, and are presented as maximum changes averaged over about five breaths; control measurements were averaged over 5-10 breaths. All these tests were repeated after partial and after complete vagal block.

The Student paired t-test was used to assess changes in each variable produced by drugs or by hypercapnia. All results are given as means ± SEM.

RESULTS

Control values

Mean control value of Rlar in intact animals was initially 0.069 ± 0.0070 kPa · l^{-1} · s; there were modulations of translaryngeal pressure with the breathing rhythm, the maximum resistance being during expiration [23]. Control Rlar tended to increase throughout the experiments. Mean control value of RL was 0.55 ± 0.024 kPa · l^{-1} · s. These values were obtained from 20 s periods sampled before each test (49 periods in nine dogs). Thus Rlar was about one-tenth RL, corroborating values obtained by STRANSKY *et al.* [23] for cats and rabbits.

After completion of partial vagal block, RL increased slightly to 0.62 ± 0.025 kPa · l^{-1} · s, as did Rlar to 0.094 ± 0.0067 kPa · l^{-1} · s. These changes could be partly the result of chest surgery. Complete vagal block did not produce any further change in Rlar but lowered the mean value of RL to 0.48 ± 0.019 kPa · l^{-1} · s. All these changes were significant compared to values in neurally-intact dogs ($p < 0.01$). The effects of vagal block on control Vtr were not determined.

Responses to histamine i.v.

Mean changes in RL, Vtr and Rlar are given in table I and figure 2. Histamine induced large increases in RL (+ 950 %) and Rlar (+ 1160 %) and decreased Vtr. After partial vagal block or complete vagal block, all responses to histamine i.v. were virtually abolished and statistically insignificant.

Responses to histamine aerosol

Mean changes in RL, Vtr and Rlar are given in table I and figure 3. In vagus-intact dogs, histamine aerosol had very similar actions to histamine i.v., increasing RL (+ 1084 %) and Rlar (+ 1668 %), and decreasing Vtr. In dogs with partial vagal block, the effect of histamine aerosol on RL was about halved, whereas the effects on Rlar and Vtr were virtually abolished and statistically insignificant. After complete vagal block, histamine aerosol had no measurable effects on any of the three variables.

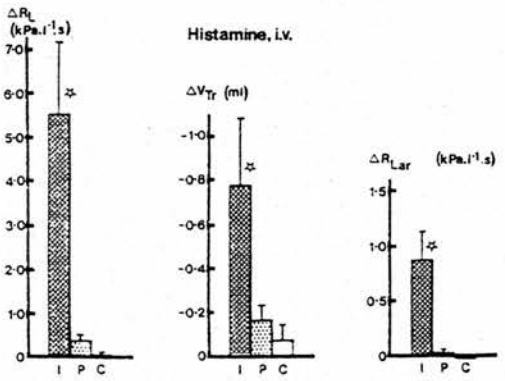

Fig. 2. — Effects of intravenous histamine on RL, Vtr and Rlar, in dogs vagally-intact (I), with partial vagal block (P) and with complete vagal blockade (C). Results are given as changes from control. * $p < 0.05$, as in table I.

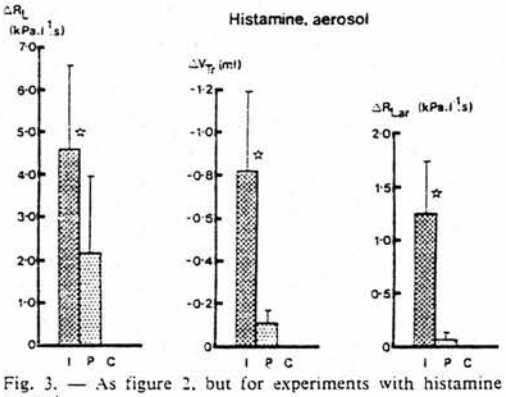

Fig. 3. — As figure 2, but for experiments with histamine aerosol.

Table I. — Changes in RL, Rlar and Vtr caused by various stimuli

	Condition	n/N	RL Change (kPa · l^{-1} · s)	n/N	Vtr Change (ml)	n/N	Rlar Change (kPa · l^{-1} · s)
Histamine i.v.	VI	10/7	+ 5.55 ± 1.62*	10/7	− 0.78 ± 0.29*	10/7	+ 0.87 ± 0.31*
	PVB	4/4	+ 0.33 ± 0.17	4/4	− 0.16 ± 0.067	4/4	+ 0.03 ± 0.02
	CVB	5/5	− 0.062 ± 0.062	5/5	− 0.072 ± 0.072	5/5	− 0.016 ± 0.016
Histamine aerosol	VI	8/8	+ 4.60 ± 1.97*	9/9	− 0.82 ± 0.37*	9/9	+ 1.25 ± 0.49*
	PVB	5/5	+ 2.17 ± 1.79	5/5	− 0.11 ± 0.056	5/5	+ 0.072 ± 0.072
	CVB	3/3	0	3/3	0	3/3	0
Capsaicin i.v.	VI	14/9	+ 1.37 ± 0.63*	13/9	− 0.49 ± 0.14*	14/9	+ 0.52 ± 0.21*
	PVB	5/5	+ 0.28 ± 0.11*	5/5	− 0.21 ± 0.067*	5/5	+ 0.0098 ± 0.0077
	CVB	8/8	− 0.017 ± 0.017	7/7	− 0.061 ± 0.044	7/7	0
Hypercapnia	VI	17/8	+ 0.25 ± 0.049*	22/9	− 0.25 ± 0.050*	22/9	− 0.13 ± 0.055*
	PVB	8/5	+ 0.45 ± 0.24*	8/5	− 0.26 ± 0.050*	8/5	− 0.0062 ± 0.0027*
	CVB	9/9	+ 0.085 ± 0.025*	9/9	− 0.18 ± 0.067*	9/9	+ 0.0020 ± 0.0020

Values are means ± SEM and apply to numbers of tests. VI : vagus-intact ; PVB : partial vagal blockade ; CVB : complete vagal blockade ; n : number of tests ; N : number of dogs. * $p < 0.05$.

Responses to capsaicin i.v.

Mean changes in RL, Vtr and Rlar are given in table I and figure 4. Capsaicin increased RL and Rlar, and decreased Vtr in vagus-intact dogs. In the doses used, the responses were considerably smaller than with histamine. In dogs with partial vagal block, there were still statistically significant changes in RL and Vtr, although both were reduced in size. During complete vagal block, responses of all three variables were virtually abolished and statistically insignificant.

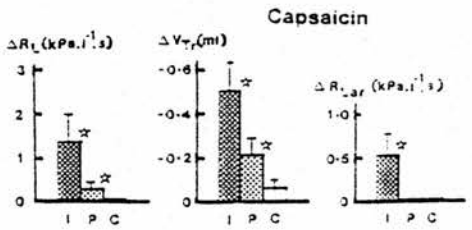

Fig. 4. — As figures 2 and 3, but for experiments with capsaicin.

Responses to hypercapnia

Mean changes in RL, Vtr and Rlar are given in table I and figure 5 : it should be noted that the scales in figure 5 differ from those in figures 2-4. Hypercapnia caused small but statistically significant increases in RL (+ 38 %) and decreases in Rlar and Vtr. After the beginning of inhalation of the CO_2-rich gas mixture, the first measurable change in Vtr was at 11.8 ± 2.9 s ; recovery started within 30-50 s after the end of the stimulus. At the same time, Rlar decreased but came more quickly back to control level when hypercapnia was stopped.

During partial vagal block, hypercapnia still increased RL and decreased Vtr as much as or more than in the vagus-intact dogs. Its dilating effect on the larynx was greatly reduced, although still statistically significant. Initial changes in tracheal volume began 18.1 ± 2.2 s after the onset of the end-tidal CO_2 increase.

During complete vagal block, the constrictor effects of hypercapnia on RL and Vtr were less than in the previous conditions, although they were still present and statistically significant. There were no significant changes in Rlar. The first measurable change in Vtr occurred at 34.6 ± 2.1 s after the beginning of the hypercapnia, and this prolongation of latency of response was significant compared to controls and partial vagal block.

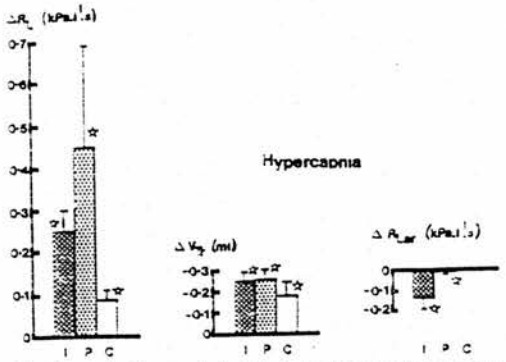

Fig. 5. — As figures 2, 3 and 4, but for experiments with hypercapnia. Note that the scales for RL and Rlar are different from those in the other figures.

DISCUSSION

Interpretation of results is complicated because of the nature of the denervations and vagal blockades. Since we wished to test the role of myelinated and nonmyelinated afferent fibres from the lungs separately, and since in practice this can only be done by differential vagal blockade, it was not appropriate to compare laryngeal and tracheal responses before and after complete lung denervation. Differential vagal blockade of the lungs is not practical at a level below the origin of the recurrent laryngeal nerves, at least on the left side. Therefore we had to block the vagus where it includes the recurrent laryngeal nerves, which inevitably would have an effect on motor innervation of the trachea and larynx. Table II summarizes the effects of the partial and complete vagal block. It will be seen that partial vagal block includes : 1) abolition of all myelinated afferent conduction from the lungs ; 2) retention of half the nonmyelinated afferent conduction from the lungs ; 3) block of all motor innervation to the lungs ; and 4) block of half the motor innervation to the cervical trachea and larynx. These conclusions are based upon the belief that cold block to 5 °C of the cervical vagus nerve will inhibit conduction both in afferent and in efferent myelinated fibres [6, 15, 25] while leaving that in nonmyelinated afferent fibres intact ; and that the motor innervation to the larynx and upper trachea via the superior laryngeal nerves [5] is quantitatively small compared to that via the recurrent laryngeal nerves.

Table II. — Effects of denervations

	Afferents from lungs		Efferents to lungs	Efferents to trachea	Efferents to larynx
	myelinated	nonmyelinated			
Vagi intact	intact	intact	intact	intact	intact
Partial vagal block	blocked	halved	blocked	halved	halved
Complete vagal block	blocked	blocked	blocked	halved	halved

A further complication is that the test stimuli to the dogs might have not only primary reflex actions on the airways, which was the impetus for the study, but also secondary effects via blood gas changes and changes in pattern of ventilation, which would activate reflexes such as that from pulmonary stretch receptors [8]. The last reflex is known to cause bronchodilatation [22, 24, 25] and to move the laryngeal walls to partial abduction [23]. To study the responses to stimuli in dogs with muscular relaxants would have obviated an important part of the study, namely that of laryngeal calibre which depends upon striated muscles. However, despite these reservations, some semiquantitative conclusions can be drawn.

Histamine

In vagus-intact animals, histamine, intravenously or by aerosol, causes large increases in RL and Rlar, and tracheal constriction [21, 23]. These effects were greatly reduced or abolished by partial or complete vagal block. This confirms the view that intravenous histamine is acting mainly on irritant receptors with myelinated afferent fibres [21], and leaves only a small role for C-fibre receptors. Stimulation of lung receptors with histamine causes tracheobronchial [6] and laryngeal [12, 23] constrictions, and both irritant receptors and C-fibre receptors can be stimulated by the drug [1, 6].

With histamine aerosol, the results were essentially similar, except that with partial vagal block the drug still caused a considerable increase in RL. Although this might be due to histamine by this route having a direct action on bronchial smooth muscle, one would not have expected the response to be abolished by complete vagal block had this mechanism applied.

Capsaicin

The results with capsaicin were qualitatively similar to those with histamine. In vagus-intact dogs, the drug increased RL and Rlar and constricted the trachea [6, 9, 20]. As with histamine, partial vagal block reduced all the responses, while complete vagal block virtually abolished them.

Although capsaicin, in minimal intravenous doses, is assumed to be a 'specific' stimulant of bronchopulmonary C-fibre receptors, some drugs that act on these receptors are known also to affect irritant endings [1, 6]. Similarly, histamine is sometimes claimed to have a more powerful or even specific action on irritant receptors [21], but is also known to stimulate C-fibre endings [6]. C-fibre stimulation causes bronchoconstriction in cats [12, 17] and dogs [8, 9, 20] and laryngeal constriction in cats and rabbits [14, 23].

Our results do not allow any distinction between the roles of irritant and C-fibre receptors in influencing RL, Rlar and Vtr, and are compatible with both groups of receptors having qualitatively similar effects on all three parts of the airway. Indeed, both types of receptors respond similarly to a wide variety of irritant, chemical and pathological stimuli [1, 6], and it seems quite likely that both groups of receptors exert additive influences in many pathophysiological conditions.

Hypercapnia

In the vagus-intact dogs, hypercapnia caused a small increase in RL, a tracheal constriction and a laryngeal dilatation. These results confirm other published work [2, 12, 14, 16, 19, 23]. During partial vagal block, all three effects were still present, although the laryngeal change was greatly reduced. As explained above, one would have expected the denervations to have abolished the lung response and halved the tracheal and laryngeal responses simply by blockade of motor nerves. However, other studies have shown that hypercapnia increases RL after vagotomy [16, 24]; the mechanism of this is not clear. Complete vagal block still left significant changes in RL and Vtr, but now the laryngeal response was abolished. We conclude that, although hypercapnia inhibits the discharge of pulmonary stretch receptors [4, 6], increases the activity of bronchopulmonary C-receptors [1, 7, 11] and possibly inhibits the action of rapidly adapting irritant receptors [7], these reflex mechanisms do not exert appreciable actions on the airways in our experimental conditions. Indeed, were the stimulation of C-fibre receptors important, one would expect complete vagal block to enhance the dilator effect of hypercapnia on the larynx rather than diminish it, since a constrictor reflex would be abolished. It must be concluded that the dominant effect of hypercapnia on the airways is on the central chemoreceptors in the brainstem as is true also for the control of breathing, and that reflex modulation via lung receptors is small or absent.

As indicated in the Introduction, there is some difference of opinion as to the importance of the role of vagal afferent fibres in CO_2-induced bronchoconstriction. In cats, the influence of vagal reflexes is claimed to be strong [12, 17], whereas in dogs indirect evidence has suggested that vagal reflexes are not important [13, 19]. Our results with dogs show that partial vagal block (of myelinated fibres) had little effect on the responses of RL and Vtr to hypercapnia, while complete vagal block (including nonmyelinated fibres) reduced the response of RL but not greatly of Vtr. Thus nonmyelinated fibres may contribute to the bronchial but not the tracheal response to hypercapnia. With regard to the larynx, species difference is apparent from the results of BARTLETT [3], who found that in cats vagotomy had little effect on the laryngeal response to hypercapnia; in our results, partial and complete vagal block in the dog virtually abolished the effect of hypercapnia on Rlar.

Quantitative interpretation of results is difficult because the two patterns of denervation would change baseline conditions, partly by the denervations themselves, and also because the dogs had to undergo open chest surgery for the partial vagal block. The slow, deep breathing caused by partial and complete vagal block would change both the motor control of the larynx and the mechanical responsiveness of the lungs to induced motor drives. For this reason, an extensive statistical analysis of the results does not seem justified, and the conclusions to be drawn from the experiments are tentative and rather qualitative. For example, after complete vagal block hypercapnia should still stimulate central and

possibly peripheral chemoreceptors, and this could cause a nervous decrease in Vtr. The reduction of the change in RL and the abolition of the decrease in Rlar are more difficult to explain. However, the lung reflexes stimulated by histamine and capsaicin cannot be responsible for the laryngeal effects of hypercapnia (since it was a decrease in Rlar) or the decrease in Vtr, and the role of nonmyelinated fibre lung reflexes in these conditions seems to be limited to a possible action on the lower airways. A further complication is that hormonal influences such as release of adrenalin may influence responses of smooth muscle, but this cannot explain the difference between changes in Vtr and RL after complete vagal block. The direct action of CO_2 on airway smooth muscle has also to be considered. Although *in vitro* its action is relaxant [24], *in vivo* the extrinsically denervated lung may respond by a constriction to hypercapnia [16], and our results are consistent with the existence of this mechanism.

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RESUME : Chez 9 chiens lévriers, anesthésiés par un mélange chloralose-urethane, la résistance pulmonaire totale, le volume d'un segment isolé de trachée cervicale et la résistance à l'écoulement offerte par le larynx isolé ont été simultanément mesurés et leurs modifications étudiées dans trois circonstances : 1) innalation d'un mélange enrichi en CO_2, 2) injection intraveineuse ou innalation d'une solution d'histamine, afin de stimuler essentiellement les récepteurs pulmonaires sensibles à l'irritation ; 3) injection intraveineuse de capsaïcine pour stimuler principalement les récepteurs pulmonaires connectés avec des fibres vagales amyéliniques (fibres C vagales). Ces stimulations ont été étudiées chacune dans trois circonstances : 1) innervation des voies aériennes intacte ; 2) dénervation du poumon droit associée à un blocage par le froid du niveau cervical des fibres myélinisées contenues dans le nerf vague gauche ; et 3) blocage supplémentaire des fibres C contenues dans ce nerf. L'histamine et la capsaïcine augmentent les résistances pulmonaire et laryngée et réduisent le volume du segment tracheal. L'analyse de ces reponses aprés dénervations successives montre que ces effets resultent de la stimulation des réflexes vagaux pulmonaires. Chez l'animal intact, l'hypercapnie augmente la résistance pulmonaire totale, réduit le volume du segment tracheal et diminue la résistance laryngée. Après blocage de la conduction au niveau des fibres vagales myélinisées, les modifications de résistance pulmonaire et du volume trachéal induites par l'hypercapnie persistent, mais la dilatation laryngée produite par le CO_2 est très réduite. Le blocage de toutes les fibres vagales abolit la réponse laryngée résiduelle, diminue et retarde l'apparition des variations de volume trachéal et réduit de façon importante l'accroissement de résistance pulmonaire induit par l'hypercapnie. Ces résultats indiquent que la réponse laryngée à l'hypercapnie necessite l'intégrité des réflexes vagaux, mais que l'effet constricteur du CO_2 sur la trachée et les voies aériennes inférieures dépend moins de l'innervation vagale.

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©A ROLE OF PULMONARY RAPIDLY ADAPTING RECEPTORS IN CONTROL OF BREATHING

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Summary. We analysed the breathing pattern of anaesthetised rabbits during unloaded breathing when breathing was accelerated by inspired CO_2 and when they breathed against positive or negative pressures before and during block of pulmonary stretch receptors by SO_2, and after bilateral vagotomy. Before block moderate steps of inflation or deflation (0·5 kPa) produced relatively larger changes in duration of expiration than in duration of inspiration, indicating the relative sensitivities of the two phases. With stretch receptors blocked inflation or deflation shortened expiration, demonstrating the influence of rapidly adapting receptors on that phase of breathing. If pulmonary stretch receptors were the major determinants of the duration of inspiration, we would have expected inspiratory duration in the stretch receptor blocked and vagotomised states to be almost identical. They were not, inspiratory duration being less in the blocked than in the vagotomised state. Possibly vagal afferent activity other than that of stretch receptors shortens inspiratory duration. However, we have found that rapidly adapting receptor activity (and any unmyelinated fibre activity provoked by rapid inflation or deflation of the lungs) never directly shortened inspiration. We therefore propose a mechanism whereby rapidly adapting receptors may indirectly affect duration of inspiration.

INTRODUCTION

In the most commonly agreed theory of control of breathing, lung stretch receptors (Adrian, 1933) play the major role in providing afferent vagal information to the respiratory control centres of the brain. This theory, drawing on the work of many earlier investigators but largely unified by the work of Clark and von Euler (1972), suggested that volume-sensitive stretch receptor activity terminated the duration of inspiration (t_I) by activating an 'off switch' mechanism which brings inspiratory activity to an end. Pulmonary stretch receptors (p.s.r.) have also been implicated in the control of expiratory duration (t_E) (Bartoli *et al.*, 1973; Knox, 1973).

Tests frequently used to determine the level of afferent vagal activity from lung receptors and its effect on pattern of breathing include increasing minute

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Abbreviations used in this paper: t_I, Duration of inspiration; t_E, Duration of expiration; V_T, Tidal volume; p.s.r., Pulmonary stretch receptors; r.a.r., Rapidly adapting receptors.

ventilation by administering hypercapnic gas mixtures (Bradley *et al.*, 1974; Winning and Widdicombe, 1976) and causing the subject to breathe against a positive or negative pressure, which increases or decreases the lung volume against which respiratory excursions take place (Breuer, 1868; Clark and Euler, 1972; Knox, 1973). The duration of the two phases of respiration in relation to each other and to tidal volume (V_T) is often analysed in the interpretation of these tests.

If pulmonary stretch receptors are the major vagal determinant of the pattern of breathing under the circumstances of these tests, a block of their activity should produce a breathing pattern almost identical to that seen in vagotomised animals. To see if this is so, we have used a highly specific method of blocking stretch receptors (Davies *et al.*, 1978) to investigate their role in control of duration of inspiration and expiration in anaesthetised rabbits, in which breathing was stimulated by carbon dioxide or took place against positive or negative pressure.

Materials and Methods

We used 7 New Zealand White rabbits weighing between 2·0 and 3·5 kg. Anaesthesia was induced and maintained with sodium pentobarbitone (40 mg. kg Nembutal, Abbott). A polyethylene cannula was tied into the trachea and polyethylene catheters tied into a femoral artery and vein. Blood pressure was monitored from the arterial catheter.

Tidal volume was measured by electronically integrating air flow recorded by a Fleisch pneumotachograph head connected to the tracheal cannula. The non-leaky integrator was manually balanced for minimal drift throughout the experiment and provided a true record of tidal volume when directly calibrated at the end of the experiment.

Phrenic activity was recorded from multifibre strands of the upper root of the right phrenic nerve placed in a trough of liquid paraffin. The action potentials were picked up by two platinum electrodes and amplified electronically. Initially, phrenic activity was integrated using a 'non-leaky' integrator (Davies and Wise, 1978). The phrenic integral was used to define t_I and t_E on the basis that t_I is the interval between the initial increase and levelling off of the phrenic integral, t_E being the interval between the end of one inspiratory duration and the beginning of the next. It was found that the two points defined on the phrenic integral coincided with the onset and rapid decline of the 'raw' phrenic activity and this was used with equal convenience to define inspiratory duration. The validity of these definitions has been discussed by Widdicombe and Winning (1974). Breathing was accelerated by causing the rabbits to breathe 8% CO_2 in air passed through a T-piece connected to the pneumotachograph.

In 5 rabbits rapid inflation or deflation of the lungs was achieved by connecting the tracheal cannula, by means of a solenoid operated valve, to a large (20 litres) drum maintained at 0·5 k.Pa (5 cm H_2O) positive or negative pressure relative to atmosphere. Inflation was carried out in phase with the rabbits' inspiration and deflation in phase with expiration to provide a rapid but smooth change in lung volume. Pressure was maintained for eight breaths and released, either in expiration (positive pressure) or inspiration (negative pressure). Pulmonary stretch receptor activity was blocked by causing the anaesthetised rabbit to breathe 200 parts per million SO_2 in air for 10 min (Davies *et al.*, 1978). Abolition of the Hering-Breuer inflation reflex to a lung inflation of 1·5 k.Pa (15 cm. H_2O) was taken as evidence of p.s.r. block. Although the duration of exposure to SO_2 was not critical to our conclusions, in none of our rabbits was it necessary to increase the time of exposure to fulfill the criterion of full functional block of stretch receptors.

Bilateral cervical vagotomy was carried out after leaving the nerves in contact with 2% Lignocaine for 3 min. Five minutes were allowed after cutting the vagi before further tests were made. The variables in each experiment were recorded on a Devices chart recorder.

RESULTS

Inhalation of CO_2 caused an increase in tidal volume (V_T) and frequency of breathing. The V_T-t_I relationship for each rabbit is shown in Fig. 1. After p.s.r. block by SO_2 the curve was shifted to the right, as shown. For each value of V_T, t_I was longer than before SO_2. Vagotomy produced a further shift to the right of the V_T-t_I curve.

In 6 rabbits of the 7 the V_T-t_E relationship before and after p.s.r. block lay on a single curve (Fig. 2). In rabbit six there was a shift of the curve similar

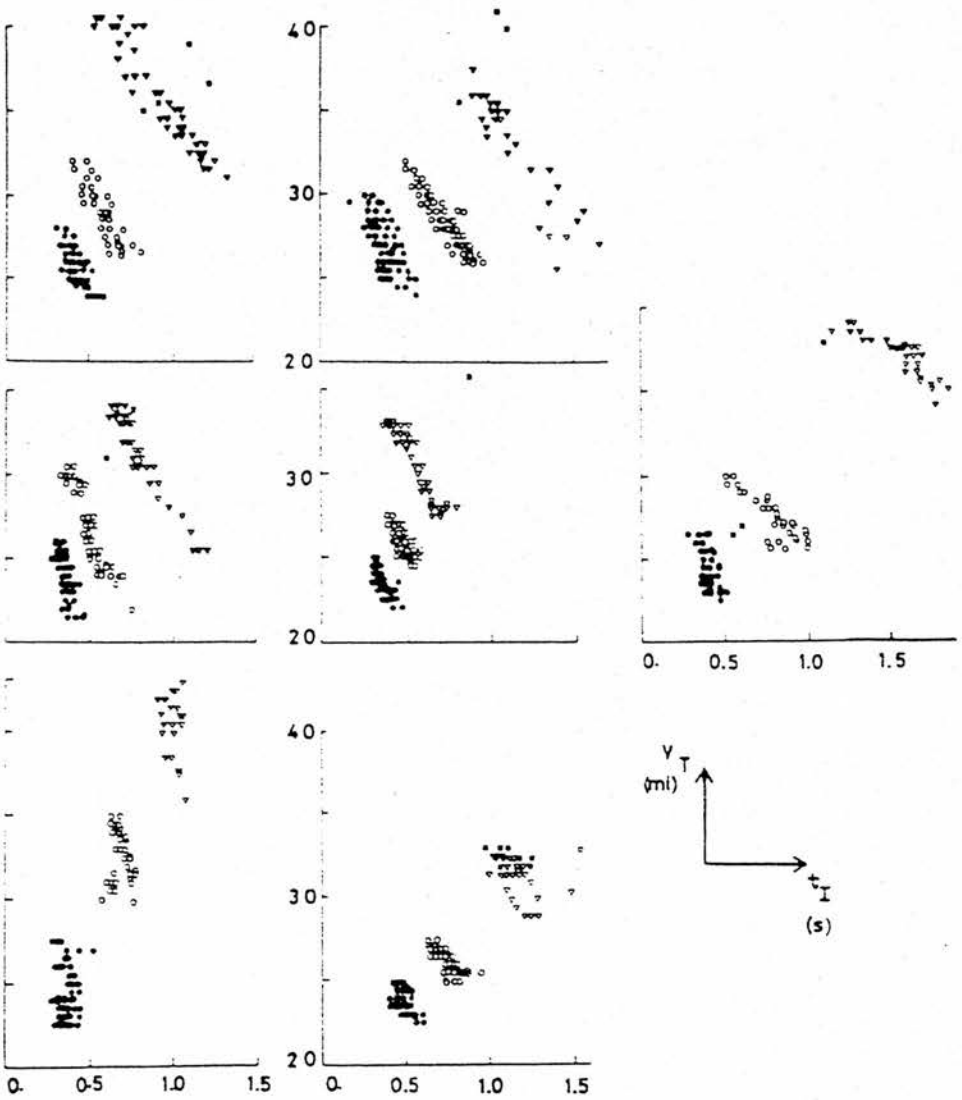

Fig. 1. The relationship between inspiratory duration (t_I) and tidal volume (V_T) in seven anaesthetised rabbits with pulmonary stretch receptors intact (●) blocked (○) and after vagotomy (▽) when breathing was stimulated by carbon dioxide. Augmented breaths shown with stretch receptors intact (■) or blocked (□) did not occur after vagotomy.

to that seen in the V_T-t_I relationship. After vagotomy the curve shifted to the right.

Steps of inflation and deflation

(a) Steps of inflation

When the rabbit's lungs were inflated by positive pressure for a period of eight breaths, with p.s.r. intact, there was an increase in t_E for as long as the positive pressure was maintained. When the pressure was returned to

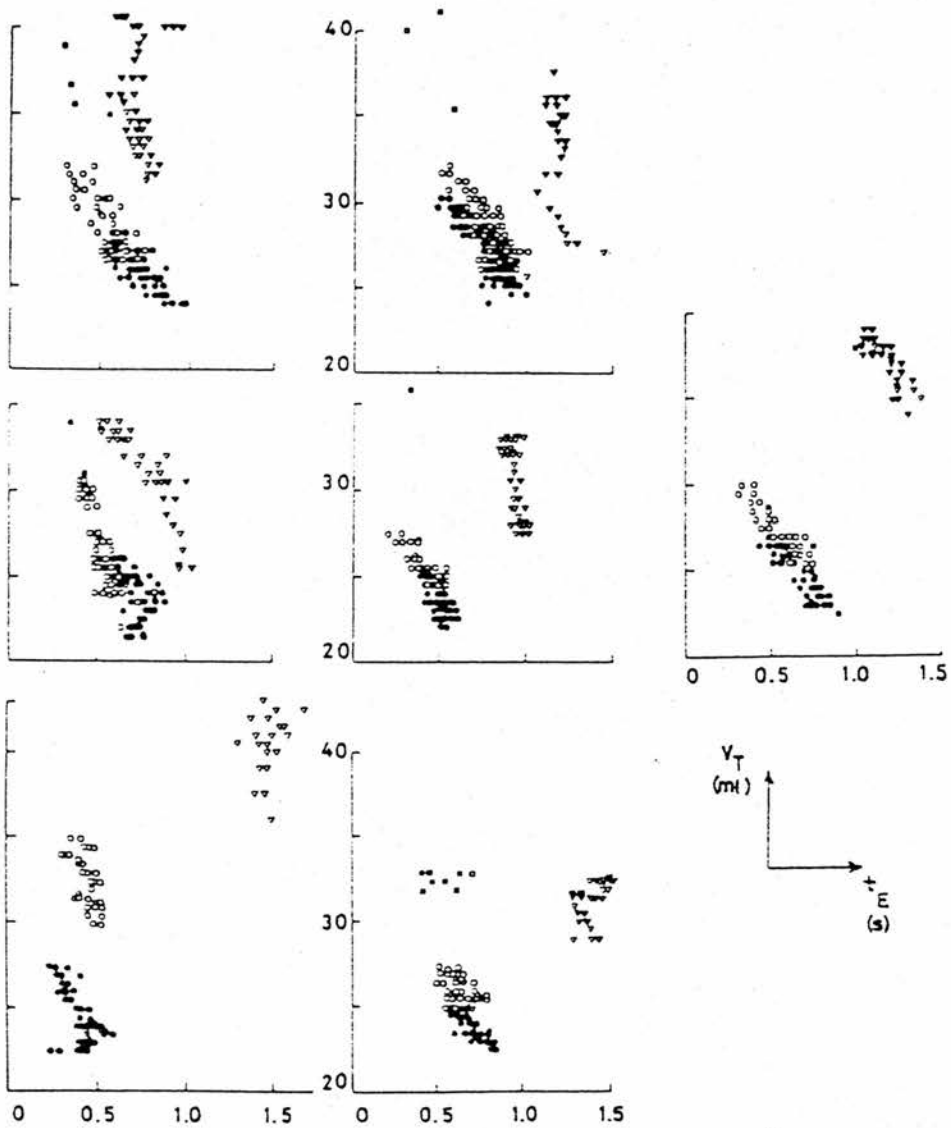

Fig. 2. The relationship between expiratory duration (t_E) and tidal volume (V_T) in seven anaesthetised rabbits with pulmonary stretch receptors intact (●) blocked (○) and after vagotomy (▽) when breathing as stimulated by carbon dioxide. Augmented breaths, shown with stretch receptors intact (■) or blocked (□) did not occur after vagotomy.

atmospheric there was an immediate decrease in t_E to below control (Fig. 3). It then returned slowly towards the control value.

When p.s.r. were blocked the step of inflation produced a decrease in t_E which was maintained as long as the positive pressure was maintained (Fig. 3). When the pressure was returned to atmospheric t_E returned to control value with much the same time course as before SO_2 block.

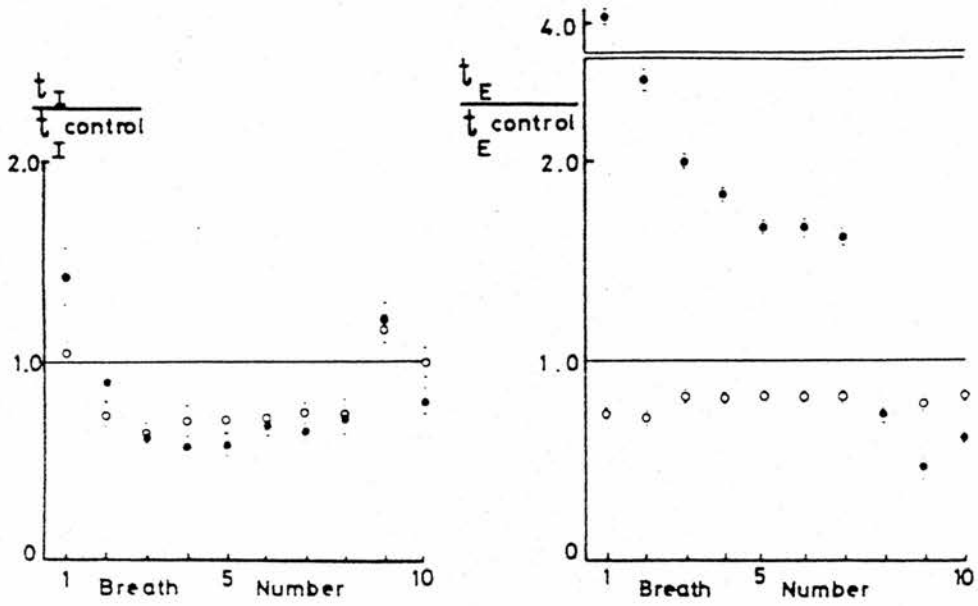

Fig. 3. Mean duration (± S.E.M.) of inspiration (t_I) and expiration (t_E) relative to a control breath, taken as 1, in five anaesthetised rabbits with stretch receptors intact (●) or blocked (○) during sustained inflation of the lungs with a positive pressure of 0·5 kPa. Inflation was in phase with inspiration of breath 1 and was released in phase with expiration of breath 8. In breath 1, 7 out of 23 inflations produced augmented breaths in the intact state and one inflation produced an augmented breath in the blocked state. t_E of breath 1 was increased when stretch receptors were intact whether t_I was augmented or not. Absolute values in the control state (t_I = 0·4$_5$ ± 0·04; t_E = 0·7$_5$ ± 0·04; N = 23) differ from the blocked state (t_I = 0·7$_5$ ± 0·09; t_E = 0·6$_5$ ± 0·03$_5$ N = 17).

The change in t_I produced by sustained lung inflation was essentially the same in intact rabbits or those with stretch receptors blocked, and consisted of a reduction in t_I (Fig. 3). This is obscured in the first breath shown in Fig. 3 by augmented breaths (7 out of 23 breaths in the intact, and 1 out of 17 breaths in the blocked rabbits) which had t_Is of approximately 1·8× control. Bilateral cervical vagotomy abolished the effects of steps of inflation.

(b) Steps of deflation

When the intact rabbit's lungs were deflated with a maintained negative pressure (−0·5 kPa) there was a large increase in breathing frequency due to a decrease in t_E (Fig. 4). This decrease was maintained throughout the deflation. On return to atmospheric pressure there was an immediate increase in t_E to above the control value. With p.s.r. block, maintained lung deflation still produced decreases in t_E during the whole period of deflation (Fig. 4).

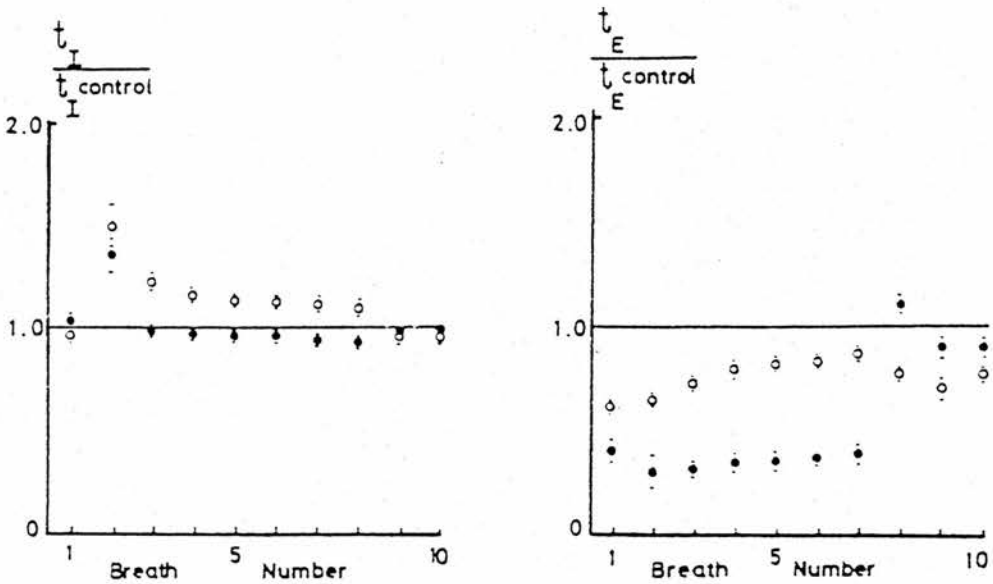

Fig. 4. Mean duration (± S.E.M.) of inspiration (t_I) and expiration (t_E) relative to a control breath, taken as 1, in five anaesthetised rabbits with stretch receptors intact (●) or blocked (○) during sustained deflation of the lungs with a sustained negative pressure of 0·5 kPa. Deflation was in phase with expiration of breath 1 and released in phase with inspiration of breath 8. In breath 1, 7 out of 21 t_I were augmented in the intact, and 7 out of 20 augmented in the blocked state. Non-augmented t_Is were not significantly different from control. ($P > 0{\cdot}05$; t-test). Absolute values in the control state ($t_I = 0{\cdot}5_4 \pm 0{\cdot}04$; $t_E = 0{\cdot}7_5 \pm 0{\cdot}03$; N = 21: Intact). ($t_I = 0{\cdot}7_5 \pm 0{\cdot}03$; $t_E = 0{\cdot}6_4 \pm 0{\cdot}02$; Blocked).

This decrease was approximately half that with p.s.r. intact. When the rabbit was returned to atmospheric pressure there was not the increase in t_E to above control value seen in the intact case, but a further small shortening, followed by a gradual return to control values of t_E with the same time course as seen with steps of inflation, both before and during receptor block.

With sustained deflation, both before and during p.s.r. block, changes in t_I were due to the triggering of augmented breaths (7 out of 21 deflations in the intact and 7 out of 20 deflations in the blocked) with extended t_Is which gradually returned to control value. Non-augmented t_Is were not significantly different from control. Bilateral cervical vagotomy abolished the effects of steps of deflation.

DISCUSSION

In the present series of experiments we have investigated some aspects of vagal afferent control of the pattern of breathing in anaesthetised rabbits. In considering our findings, we should remember that non-vagal afferent influences act on the pattern of breathing. Electrical stimulation of the splanchnic nerve in rabbits (Siegelova, 1976) and cats (Albano and Garnier, 1983) and the more physiological stimulus of urinary bladder distention (Schondorf and Polosa, 1980) inhibited phrenic activity. There is little doubt, however, that vagal afferents are a major determinant of the pattern of breathing

in the type of preparation used in the present series of experiments. Prabhaker, Marek and Loeschcke (1985), for example, suggest that visceral afferent information from the splanchnic region may only be important in pregnancy and in pathophysiological situations involving the abdominal viscera. Shannon *et al.* (1985) suggest that extra-vagal reflexes 'may not be important under the present experimental conditions' which were similar to our own. However, these extra-vagal reflexes do exist and could be important in other situations.

In the present series of experiments we produced a specific block of p.s.r. (the efficiency of block will be discussed later). The shift to the right of the V_T-t_I curve produced by p.s.r. block provides further evidence of the importance of these receptors in control of inspiratory duration in anaesthetised animals. For a given tidal volume p.s.r. block caused an increase in t_I which is in agreement with the concept of an 'off switch' mechanism. However, stretch receptor block only increases t_I to a value intermediate between the intact and vagotomised states. This could not have been due to residual stretch receptor activity because the Hering-Breuer inflation reflex was completely abolished, there being in fact a shortening of t_E. We are left with the question 'What is restricting the duration of inspiration under these circumstances of stretch receptor block?'

The V_T-t_E relationship contrasts with the V_T-t_I relationship outlined above. During p.s.r. block points describing the V_T-t_E relationship lay on a continuation of the line describing this relationship before receptor block, unlike the V_T-t_I relationship before receptor block, which was displaced by the block (Figs. 1 and 2).

Augmented inspirations were followed by expirations which fell on or close to a continuation of the V_T-t_E curve common to breathing before and during block (Fig. 2), which supports the idea that there is a unique V_T-t_E relationship that exists in the presence or absence of stretch receptor activity.

When rabbits with p.s.r. block were vagotomised the V_T-t_E curve was greatly altered. For a given V_T, t_E increased. This suggests that vagal receptors, other than p.s.r. were involved in the control of t_E. It seems likely that these receptors were rapidly adapting pulmonary receptors (r.a.r.) and we have reported in a previous paper (Davies and Roumy, 1982) that stimulation of these receptors shortens t_E.

A criticism of our use of CO_2 to stimulate breathing in these experiments might be that it depresses the activity of p.s.r. (Bradley *et al.*, 1974; Leitner, 1972; Mustafa and Purves, 1972) and so obscures the effect of a lung stretch receptor block. However, the sensitivity of receptors in the lung to CO_2 in the intact animal is low, significant sensitivity of respiratory response only being reported in experiments using pulmonary by-pass where the resting value of airway CO_2 was unphysiologically low (Bartoli *et al.*, 1974). When 4-10% CO_2 was given to animals with a normal resting airway CO_2 the decrease in p.s.r. activity was only 10-15% (Leitner, 1972). This would hardly explain why there was no shift in the V_T-t_E curve on stretch receptor block. In our experiments we must also consider the possibility of changes in pattern of breathing due to changes in depth of anaesthesia. This may in part account for the slightly

different behaviour of our individual rabbits, and to prevent these differences obliterating the effects of the experimental procedures the results are presented for individual rabbits in Figs. 1 and 2. The relationship between t_I and t_E at different V_Ts in our rabbits can be seen from Figs. 1 and 2 or can be expressed separately as a t_I-t_E plot which has the typical shape shown in Fig. 5.

Sustained lung deflation produced a large increase in breathing frequency in our rabbits, due to a decrease in t_E. This reflex has been previously attributed to a decrease in p.s.r. activity (Knox, 1973) and even to the postulated respiratory accelerating effects of low frequency p.s.r. activity (Paintal, 1973 for review). However, this increase in frequency persisted during p.s.r. block and was therefore not solely due to the activity of p.s.r. (Fig. 4). The transient increase in mean t_I immediately on deflation of the lungs (Fig. 4) was due to the triggering of seven augmented breaths in the 21 deflations in the intact, and in the 20 deflations in the blocked state. These augmented breaths had

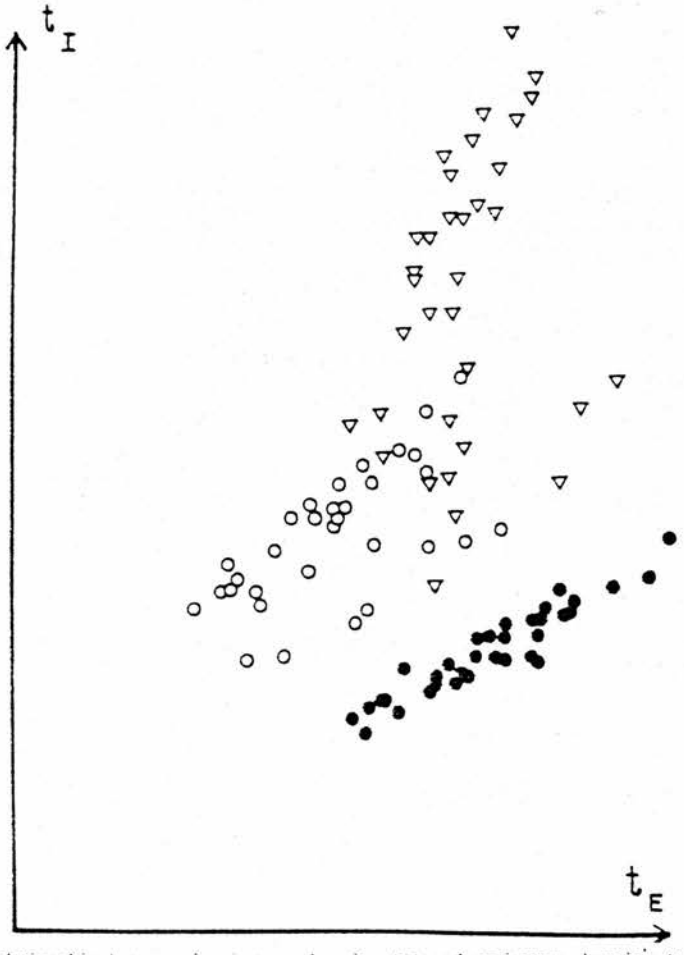

Fig. 5. The relationship between inspiratory duration (t_I) and expiratory duration (t_E) in a typical anaesthetised rabbit (No. 1) with pulmonary stretch receptors intact (●) blocked (○) and after vagotomy (▽) when breathing was stimulated by carbon dioxide.

extended t_Is (1·7×control, intact; 1·9×control, blocked). Non-augmented breaths had t_Is which were not significantly different from control. The extended t_I of the augmented breath gradually returned to control value as shown in Fig. 4.

Block of pulmonary stretch receptors and their reflex effects by SO_2 was demonstrated by the absence of the Hering-Breuer reflex seen during a step of lung inflation. We have also demonstrated the effectiveness of block by SO_2 by direct recording reported in an earlier paper (Davies *et al.*, 1978). Recently, other workers (Davenport, Freed, and Rex, 1984) have independently demonstrated the effectiveness of SO_2 block of intrathoracic p.s.r. in their investigation of these receptors as a peripheral control of t_E. Even more recently, Citterio, Piccoli and Agostoni (1985) have demonstrated SO_2 to be equally effective in blocking stretch receptors in the extra-thoracic trachea. We (Davies *et al.*, 1978) also reported no change in end-tidal pCO_2 when stretch receptors were blocked by SO_2. The Hering-Breuer reflex manifests itself as an extension of t_E, and this extension was converted to a shortening on inflation of the lungs when p.s.r. were blocked (Fig. 3). We have reported in an earlier paper (Davies and Roumy, 1982) that lung inflation causes an increase in r.a.r. activity which would produce such a decrease in t_E and which would not be affected by SO_2. We must conclude, therefore, that in the intact animal the increase in p.s.r. activity produced by a step of inflation overcame the t_E shortening r.a.r. activity. When p.s.r. were blocked, r.a.r. activity became the major afferent influence and shortened t_E.

During lung deflation the persistence of t_E shortening during p.s.r. block suggests that about 50% of this shortening was due to r.a.r. activity. We therefore suggest that expiration is controlled by a balance of stretch and rapidly adapting receptor activity. Our results from rabbits whose breathing was accelerated by CO_2 before and during p.s.r. block suggests that control of t_E is, to a large extent, carried out by r.a.r. while the results of steps of inflation and deflation indicate that p.s.r. also have a role.

The difficulty in assessing, quantitatively, the contribution made by these two groups of receptors lies in part in the different control patterns of breathing in the p.s.r. intact and blocked states which may interact with a non-linear receptor activity-reflex response relationship and so defeat quantification.

What then is the effect of r.a.r. activity on the duration of inspiration? And why, if stretch receptors are the major determinants of t_I, does vagotomy performed on an animal in which p.s.r. have been blocked shift further the V_T-t_I curve towards longer t_Is? In other words, what is preventing t_I assuming vagotomy values when p.s.r. activity is abolished and unmyelinated fibres are unlikely to be influencing the pattern of breathing at the lung volumes existing in our experiments? We might conclude that r.a.r. (not blocked by SO_2) have a direct t_I shortening effect identical to that of p.s.r. This would also explain the shortening of t_I in response to histamine aerosols and injections seen in other experiments (Davies and Kohl, 1982). However, when we stimulated r.a.r. (and unmyelinated fibres if we assume them to be activated) with pulses of inflation or deflation (Davies and Roumy, 1982) we never produced a shortening of t_I.

We produced a large increase in t_I (an augmented breath) or no effect at all. We can not therefore subscribe to the idea of a direct t_I shortening effect of r.a.r. activity, or any unmyelinated fibre activity, provoked by the manoeuvre.

In a previous paper (Davies and Roumy, 1982) we have reported that when t_E was shortened by r.a.r. activity there was a shortening of the following t_I. This took place whether p.s.r. were blocked or intact. Karczewski *et al.* (1976) reported that in the spontaneously breathing vagotomised rabbit electrical stimulation of the vagi produced decreases in t_I and t_E. The changes in t_E lead the changes in t_I (the t_I of the first cycle to contain the stimulus was not changed while t_E was reduced). When the stimulus was switched off the decrease in t_I continued for one more cycle, while t_E immediately began to return to control value. This suggests that a respiratory cycle (considered as an inspiration followed by an expiration) is not independent of the preceding cycle. There appears to be a central t_I-t_E linking which works between both phases, i.e., the duration of any t_I influences the duration of the following t_E and the duration of any t_E influences the duration of the following t_I. However, we (Davies and Kohl, 1982) have demonstrated that the linking of t_I and t_E is not mandatory and may be associated with r.a.r. activity.

By postulating a link between t_I and t_E we can explain the control of t_I at the various stages of our experiments, without evoking the concept of a direct terminating influence of r.a.r. activity on the duration of inspiration. In the intact rabbit t_I was controlled by p.s.r. activity, and t_E by a balance of p.s.r. and r.a.r. activity. When p.s.r. were blocked there was an increase in t_I and V_T. Since p.s.r. activity had been removed, t_I should have assumed its vagotomy value if p.s.r. activity was its only modulator. However, there was a shortening of t_E due to r.a.r. activity and the block of p.s.r. We postulate, because of the linking of t_I to t_E, the increase in t_I was limited to a value fixed by the central t_I-t_E relationship and the duration of t_E. Our theory predicts:-

1. Points describing the t_I-t_E relationship of individual breaths in vagotomised and p.s.r. blocked rabbits will lie on a common curve which represents the central t_I-t_E relationship.

2. Points describing individual breaths in the intact rabbit will lie away from this curve.

Both these conditions are seen in Fig. 5.

We conclude that the control of duration of inspiration and expiration of our experiments was based on a central pattern generator where frequency was probably largely determined by blood gas tensions. This relationship provided a basic linking of t_I and t_E as shown in Fig. 6. We do not define the exact nature of this relationship but it may be of the form shown. In the intact state t_I was shortened by activity from p.s.r.; t_E was lengthened by the activity of p.s.r and shortened by the activity of r.a.r. (Fig. 6a). When p.s.r. were blocked, tidal volume and t_I increased due to the removal of the restricting effect of p.s.r. activity. The major modulation of t_I was then by the central t_I-t_E relationship. Since t_E had been reduced by the removal of p.s.r. activity and r.a.r. activity increased by an increase in V_T, t_I was still restricted to less than its vagotomy value. Under these conditions the point representing

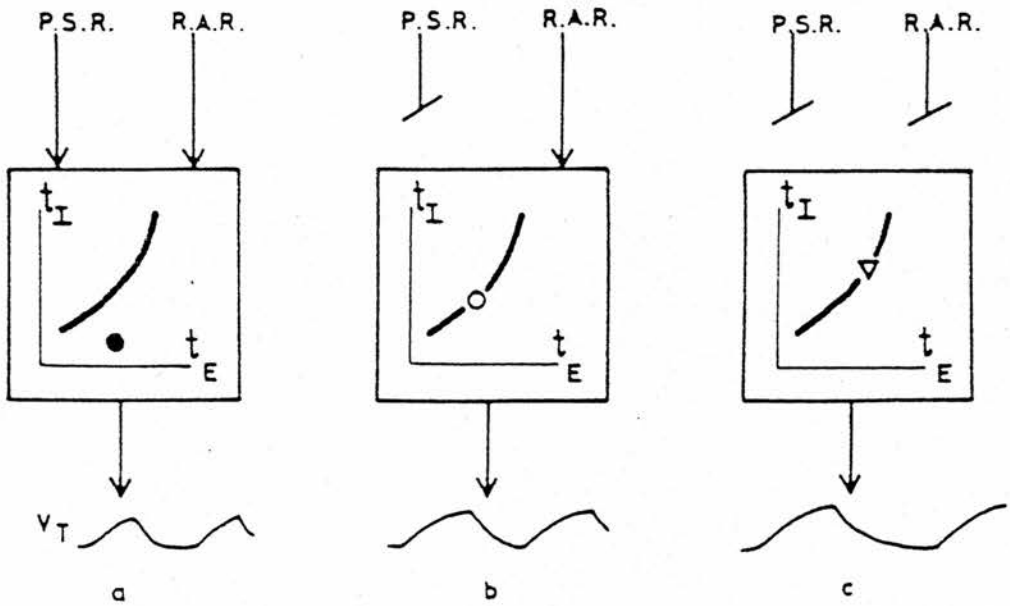

Fig. 6. The way in which duration of inspiration (t_I) may be controlled by a central relationship between t_I and t_E (expiratory duration) and vagal afferent activity in the intact (●) stretch receptor blocked (○) and vagotomised (▽) states. P.S.R.= pulmonary stretch receptor activity. R.A.R.= pulmonary rapidly adapting receptor activity. Pattern of breathing produced in each case is represented by the tidal volume (V_T) trace.

the pattern of breathing is shown in Fig. 6b. After vagotomy removed the t_E restricting effects of r.a.r. the point representing the pattern of breathing moved on the central relationship to a position solely determined by the central pattern generator (Fig. 6c).

Our findings also reconcile the two apparently opposite effects on the pattern of breathing attributed to r.a.r. activity—

1. The production of augmented breaths with prolonged t_I and—
2. The production of rapid shallow breathing with shortened t_I.

In an earlier paper (Davies and Roumy, 1982) we demonstrated that in rabbits 'After an augmented breath (provoked by stimulating r.a.r.) it was impossible to produce another for at least 1 minute,' due to the occurrence of a refractory period. Thus, during a period of intense r.a.r. stimulation such as in pneumothorax or histamine injection, a single lengthening of t_I to produce an augmented breath may take place, but after this has occurred and the t_I lengthening mechanism has become refractory the central linking of t_I to the now shortened t_E dominates to produce a pattern of rapid shallow breathing usually consisting of a profoundly reduced t_E and less markedly affected t_I.

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THE INFLUENCE OF VAGOTOMY ON THE RESPIRATORY EFFECTS OF INJECTED PHENYL DIGUANIDE IN ANAESTHETIZED RABBITS

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SUMMARY

The effects of intravenous injections of phenyl diguanide (PDG) on pattern of breathing were studied before, immediately after, and some time after bilateral cervical vagotomy in anaesthetized rabbits. The characteristic tachypnoea observed in the intact animals could not be produced for several minutes after bilateral vagotomy, but after 15 min could be elicited again. This sequence of events was not essentially modified by cutting the glossopharyngeal nerves nor by injection of local anaesthetic into the pericardial sac. We suggest that the respiratory effects of PDG in the rabbit, previously considered to originate mainly from peripheral receptors, including those of the lungs, has a considerable central component which has previously been obscured by the short-term effects of vagotomy.

INTRODUCTION

The pattern of breathing in rabbits is influenced by reflex mechanisms that modify the output of the respiratory centres of the brain. In attempts to elucidate these mechanisms workers have used various substances to stimulate, more or less specifically, receptors associated with afferent nerve fibres, particularly from the lungs. A large part of the reflex control of pattern of breathing has its afferent arm in the vagus nerves. Since a substantial part of these nerves is made up of non-myelinated fibres the action of phenyl diguanide (PDG), which stimulates such fibres, is of great interest as a tool to investigate reflex control of breathing.

Among the earliest workers to investigate the respiratory effects of PDG were Dawes & Mott (1950) who observed, as others did later, that the respiratory effects of PDG were probably the result of stimulation of receptors in the lungs, and that these effects were abolished by vagotomy. Since then it has been demonstrated that other non-myelinated fibres from the gut (Paintal, 1954), heart, and carotid chemoreceptors (Anand & Paintal, 1980) can be stimulated by PDG. It is therefore important when attributing actions of PDG to stimulation of receptors in the lungs to ensure that these other sources of activity are excluded. If the receptors of the carotid region are excluded one would expect all responses to intravenous injections of PDG to be abolished by cervical vagotomy, if the drug produces its effects by activation of peripheral receptors. This is what previous observations by us (Davies, Dixon, Callanan, Huszczuk, Widdicombe & Wise, 1978) and other workers have led us to suggest (Fig. 1). However, Miserocchi, Trippenbach, Mazzarelli, Jaspar & Hazucha (1978) observed that in rabbits vagotomy did not abolish the respiratory effects of injected PDG. The present study explains these different findings in terms of the transient effects of vagotomy on respiratory responses to PDG.

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Table 1. *Abstracts of some observations on the effect of PDG on pattern of breathing of rabbits after vagotomy*

Date	Authors	Observations
1950	Dawes & Fastier	'The effects in the rabbit were not completely abolished by vagotomy.'
1950	Dawes & Mott	'...the response is abolished by cutting the vagi...'.
1951	Dawes, Mott & Widdicombe (using low doses)	'...cutting the vagi abolished these respiratory changes. In a minority of rabbits...still caused same alteration in respiration...abolished by denervation of carotid region.'
1969	Karczewski & Widdicombe	'Vagotomy abolished the respiratory changes.'
1971	Guz & Trenchard	'This ventilatory response disappeared when conduction in non-myelinated fibres was abolished by section. Occasionally...the response to phenyl diguanide, mediated by receptors at the carotid bifurcation was seen after vagal section.'
1978	Davies *et al.*	'After vagotomy phenyl diguanide had no effect on pattern of breathing.'
1978	Miserocchi *et al.*	'PDG after vagotomy still caused a significant shortening of the expiratory time.'

METHODS

We used twenty-five New Zealand White rabbits of either sex weighing between 2·0 and 3·5 kg. Anaesthesia was induced and maintained with sodium pentobarbitone (40 mg/kg, Anathal, V. R. Labs., Australia). A polyethylene cannula was tied into the trachea and polyethylene catheters tied into a femoral artery and vein so that the tip of the venous catheter lay close to the right atrium.

Air flow was recorded from a Fleisch pneumotachograph head attached to the tracheal cannula. Phrenic activity was recorded from multi-fibre strands of the central cut end of the upper root of the right phrenic nerve placed in a trough of liquid paraffin; two platinum electrodes and a Neurolog pre-amplifier (N103) and amplifier (N105) were used. The durations of inspiration (T_I) and expiration (T_E) were measured from the initial increases and the starts of the rapid decreases of phrenic activity. PDG (30 μg/kg) was given via the intraveous catheter (1) to the intact rabbit, (2) after injecting xylocaine into the pericardial sac, (3) immediately after bilateral cervical vagotomy, and (4) 15 min after vagotomy and (5) after cutting the glossopharyngeal nerves near the base of the skull.

Injection of xylocaine was in the form of 1 ml of a 2% (v/v) solution given by the following method. A small nick in the mid line of the abdominal wall below the xiphisternum exposed the diaphragm through which the heart could be seen beating. A syringe containing the anaesthetic was attached to a three-way connector; the two remaining ports of the connector were connected to a hypodermic needle and a pressure transducer. The needle was advanced through the diaphragm into the pericardial sac until a cardiac pressure rhythm was detected by the transducer. The local anaesthetic was then injected. Indian ink in the local anaesthetic enabled the site of injection to be checked at post-mortem. 5 min were allowed to elapse after the injection of local anaesthetic and the Hering–Breuer inflation reflex, in response to a positive pressure of 10 cmH_2O, was tested before a further dose of PDG was given.

Bilateral vagotomy was carried out at the level of the larynx by cutting, in rapid succession with scissors, both nerves. Variables were recorded on a Gould 2400S chart recorded.

The significance of the changes due to injections of PDG was tested by the paired t test. The significance of the difference in changes produced by injections of PDG before, and after, vagotomy, glossopharyngeal nerve section and injection of xylocaine into the pericardial sac, were tested by a one-way analysis of variance. Fishers test of independence was used to test the significance of the absence of apnoea after xylocaine. Results are given as mean $\pm$S.E.M.

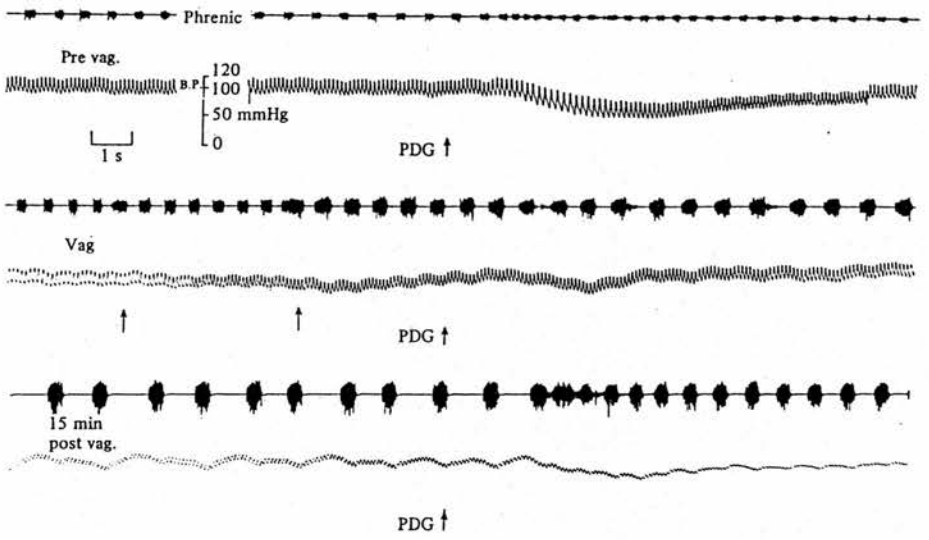

Fig. 1. The effect of intravenous injection of PDG (30 μg/kg) on phrenic discharge (upper of each pair of traces) and arterial blood pressure (lower of each pair of traces) of an anaesthetized rabbit before (pre vag.), immediately after (vag.), and 15 min after (15 min post vag.) both vagi were cut (at the time indicated by the first two arrows of the middle pair of traces).

RESULTS

In intact rabbits intravenous injection of PDG caused tachypnoea due mainly to a reduction in T_E (Fig. 1, Tables 2 and 3).

Vagal and glossopharyngeal nerve section

Bilateral vagal section produced an increase in both mean T_I ($0{\cdot}56 \pm 0{\cdot}03$ to $1{\cdot}16 \pm 0{\cdot}7$ s) and T_E ($0{\cdot}96 \pm 0{\cdot}05$ to $1{\cdot}5 \pm 0{\cdot}09$ s). The post-vagotomy pattern of breathing developed over a period of minutes, as illustrated in Fig. 2. Subsequent section of the glossopharyngeal nerve produced no significant change in T_I but a further increase in T_E ($1{\cdot}5 \pm 0{\cdot}09$ to $1{\cdot}88 \pm 0{\cdot}15$ s, $n = 5$).

Intrapericardial xylocaine

Xylocaine injected into the pericardial sac did not significantly alter the mean duration of T_I(control, $0{\cdot}56 \pm 0{\cdot}03$ s, $n = 19$; injected, $0{\cdot}63 \pm 0{\cdot}07$ s, $n = 9$) but caused a highly significant reduction in mean T_E(control, $0{\cdot}96 \pm 0{\cdot}05$ s, $n = 19$; injected, $0{\cdot}49 \pm 0{\cdot}06$ s, $n = 9$). Intrapericardial xylocaine did not abolish the effect of intravenous injection of PDG before or after section of the vagus and glossopharyngeal nerves (Fig. 2). The Hering–Breuer inflation reflex was not diminished by any of the intrapericardial injections of xylocaine.

Intravenous PDG

The respiratory effects of intravenous injection of PDG (30 μg/kg) before vagotomy, immediately after vagotomy and 15 min after vagotomy are shown in Fig. 1 and Table 2. Intravenous PDG caused tachypnoea before, 15 min after vagotomy and after cutting the

Table 2. *The effect of injected PDG on pattern of breathing of anaesthetized rabbits before bilateral vagotomy, 15 min after vagotomy, and after cutting the glossopharyngeal nerves. The lower half of the table represents rabbits which had received intrapericardial xylocaine*

	Intact		Post vagotomy		Post glossopharyngeal section	
	T_I (s)	T_E (s)	T_I (s)	T_E (s)	T_I (s)	T_E (s)
No xylocaine						
Control	0·56±0·03	0·96±0·05	1·16±0·7	1·50±0·09	1·19±0·04	1·88±0·15
PDG	0·50±0·03	0·40±0·06	1·13±0·7	0·47±0·10	1·10±0·20	0·42±0·20
	(n = 19)		(n = 18)		(n = 5)	
After xylocaine						
Control	0·63±0·07	0·49±0·06	0·87±0·10	1·85±0·33	1·20±0·3	2·57±0·37
PDG	0·58±0·07	0·24±0·04	0·85±0·20	0·74±0·23	0·99±0·2	1·19±0·17
	(n = 9)		(n = 7)		(n = 5)	

glossopharyngeal nerves, (in the presence or absence of intrapericardial xylocaine) due to a significant shortening of T_E at the 1% level (Table 2 and Fig. 1).

In all cases T_I was shortened by injections of PDG but this was only statistically significant at the 1% level in the intact state in the absence of xylocaine in the pericardial sac. Injection of xylocaine into the pericardial sac significantly reduced the response of T_E to injected PDG at the 1% level (Table 3). The response of T_I to injections of PDG was not significantly changed. PDG injected within 2 min of vagotomy did not produce a significant or consistent change in either T_E (mean change, −0·013 s) or T_I (mean change, 0·005 s) (Fig. 1). PDG injected 15 min after vagotomy produced significantly greater changes in T_I and T_E ($P < 0·05$) than before vagotomy in the presence or absence of intrapericardial xylocaine. However, the control value of T_I and T_E were themselves increased by vagotomy (Table 2) and so the changes are not strictly comparable in absolute terms.

In the intact, vagotomized and glossopharngeal nerve-sectioned cases, in the absence of intrapericardial xylocaine, forty-four injection of PDG caused apnoea in nineteen cases. In the presence of xylocaine apnoea did not occur as a result of any of twenty injections of PDG. This difference was significant at the $P = 0·01$ level.

DISCUSSION

The object of the present study was to investigate the influence of vagotomy on the effect of intravenous injections of PDG on the frequency of breathing of anaesthetized rabbits; and particularly to explain the difference between results observed by us and other workers, who found the effects of injected PDG were virtually abolished by vagotomy, and those of Miserocchi *et al.* (1978, see Table 1) who reported that vagotomy did not abolish the effects of intravenous injections of PDG.

Injection of PDG into the right atrium causes tachypnoea mainly as a result of a shortening of T_E (Dawes & Mott, 1950; Karczewski & Widdicombe, 1969; Miserocchi, Tripenbach Mazzarelli, Jaspar & Hazucha, 1978). A major part of this effect has been attributed to stimulation of type J receptors in the lungs (Karczewski & Widdicombe, 1969). The physiological stimulus of these receptors is thought to be changes in the tissue fluid

Table 3. *Mean changes in* T_E *and* T_I *in response to injected PDG and the level of significance of those changes in rabbits intact, bilaterally vagotomized, and with glossopharyngeal nerves cut, and with or without xylocaine in their pericardial sac*

	Mean change in T_E (s)	Significance (P)	Mean change in T_I (s)	Significance (P)
No xylocaine				
Intact	−0·62	<0·001	0·05	<0·05
Vagotomized	−1·09	<0·001	−0·04	>0·5
Glossopharyngeals cut	−1·46	<0·001	−0·10	>0·5
Xylocaine				
Intact	−0·24	<0·01	−0·05	>0·2
Vagotomized	−1·11	<0·01	−0·03	>0·5
Glossopharyngeals cut	−1·50	<0·01	−0·03	>0·5

surrounding the pulmonary capillaries (Paintal, 1973), changes which are not easily brought about in a controlled fashion. Also, as Sant'Ambrogio (1982) points out, these receptors generally lack any respiratory modulation, which makes their study difficult, thus PDG seemed to offer a convenient alternative method of stimulating them in order to investigate the reflex effects of their activity. However, the use of PDG in this way is not without difficulty. J receptors are associated with non-myelinated fibres in the vagus nerves. Histological investigations indicate there are substantial differences in the ratio of myelinated to non-myelinated fibres in the vagi of at least two of the species in which PDG has been used, and this may reflect different contributions to reflex control of breathing in these species. In the cat, non-myelinated vagal fibres are about three times more numerous than myelinated fibres (Agostini, Chinnock, Daly & Murray, 1957) while in the rabbit they are in a minority (Evans & Murray, 1954). However, these results, obtained with light microscopy, should be interpreted with caution as they probably underestimate the number of non-myelinated fibres. It seems also that C-fibre endings in the respiratory system are not an homogeneous group. Coleridge & Coleridge (1977) describe two groups which are distinctly different, at least on the basis of their accessibility via the bronchial or pulmonary circulation. PDG also stimulates non-myelinated fibres associated with non-pulmonary receptors, including gastrointestinal and epicardial receptors, and carotid and aortic chemoreceptors (Paintal, 1973). However, intravenous injections of PDG of less than 60 μg/kg are reported to stimulate pulmonary non-myelinated fibres but not gastrointestinal or aortic chemoreceptors (Anand & Paintal, 1980). The same authors report that local anaesthetic injected into the pericardial sac abolishes the activity of epicardial receptors, and section of the glossopharyngeal nerves will cut off activity from the carotid chemoreceptors (Chalmers, Korner & White, 1967).

If the receptors of the carotid region are excluded and the action of PDG is to stimulate the endings of the non-myelinated fibres outlined above, one would expect all response to intravenous injections of PDG to be abolished by cervical vagotomy, if this drug produces its effects by activation of these peripheral receptors. This is what we and other workers have found (Table 1). However, in 1978 Miserocchi *et al.* observed that vagotomy did not abolish the response of rabbits to injected PDG. We originally supposed this difference to be due to the anaesthetic that this group used containing little more than half the barbiturate which we used exclusively as an anaesthetic, Dawes & Mott (1950) having pointed out the powerful suppressing effect of pentobarbitone on the reflexes produced by PDG. This did

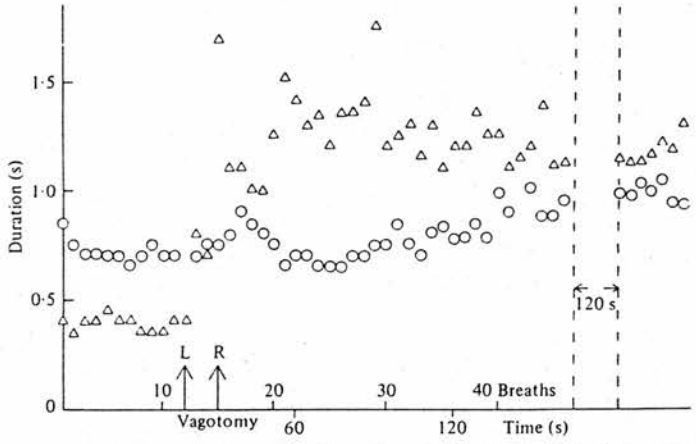

Fig. 2. The development of vagotomized pattern of breathing in a typical anaesthetized rabbit. The ordinate represents the duration of inspiration, T_I (△), and expiration, T_E (○), of sequential breaths in seconds. The abscissa represents time in seconds and number of breaths. At L and R respectively the left and right vagi were cut. At '120 s' an interval of 120 s occurs.

not prove to be the case. In the present study we have used the same level and type of anaesthetic as in our original study and have been able to confirm the observations of Miserocchi *et al.* (1978).

Section of the vagi produced an increase in mean T_I($0{\cdot}56 \pm 0{\cdot}03$ to $1{\cdot}16 \pm 0{\cdot}7$ s) and mean T_E($0{\cdot}96 \pm 0{\cdot}05$ to $1{\cdot}5 \pm 0{\cdot}09$ s). These changes, which confirm earlier findings, developed over a period of several minutes (Fig. 2) which suggests the changes of 'state' of the respiratory centres, as discussed by Feldman & Grillner (1983) are relatively slow even as a result of as rapid a procedure as section of the vagi. Section of the glossopharyngeal nerves caused a further increase in T_E. These changes in duration of T_I and T_E posed difficulties in the comparison of the effects of injected PDG before and after nerve section but they did not obscure the fact that at an appropriate interval after vagotomy (during which time the rabbit did not respond to injections of PDG) sensitivity to PDG returned.

Injection of xylocaine into the pericardial sac did not abolish the effect of injected PDG. Post mortem examination of the thoracic contents showed the Indian ink added to the xylocaine as a marker was contained in the pericardial sac. Persistence of the Hering–Breuer inflation reflex provided further functional evidence of the localization of the xylocaine.

Intrapericardial xylocaine abolished the apnoea produced by PDG injections in our rabbits. Anand & Paintal (1980) found in cats that apnoea occurred even after block of epicardial receptors by local anaesthetic. There appears to be, however, a profound species difference between cats and rabbits as far as their response to PDG is concerned. In cats, apnoea as a result of injected PDG occurs in the end-expiratory position (Winning & Widdicombe, 1976); in rabbits breathing stops in the inspiratory position. These differences may be related to the proportionally larger number of non-myelinated fibres found in the vagi of cats (Agostoni *et al.* 1957) compared to rabbits (Evans & Murray, 1954).

The distribution of receptors with non-myelinated fibres in the bronchial tree has been divided into 'bronchial' and 'pulmonary' by Coleridge & Coleridge (1977) on the basis of their circulatory accessibility, but as Sant'Ambrogio & Sant'Ambrogio (1982) point out this does not guarantee their respective locations. Neither does it imply different reflex effects

of their activity. It was not a part of the design of the present study to separate these two types of receptor.

PDG is reported to stimulate chemosensory tissue in doses higher than 60 μg/kg. Our doses should therefore have had little effect on breathing via this afferent route. The glossopharyngeal nerves were also cut to remove the afferent input from the important chemosensitive tissue of the carotid region. This does not guarantee the removal of activity from miniglomera described in this area of the cat by Matsuura (1973), and proposed to exist in the rat by Martin-Body, Robson & Sinclair (1985), which are not innervated by the carotid sinus nerve. Nor would it denervate neuroepithelial bodies in the abdomen supplied by the splanchnic nerve (Hollinshead, 1941). Chemical, mechanical and electrical stimulation of abdominal viscera have all been shown to produce changes in pattern of breathing by extra-vagal mechanisms (Frank, 1975; Chernigovisky, 1976; Mei, 1976; Prabhakar, Marek & Loeschcke, 1985). Section of the glossopharyngeal nerves in our experiments produced increases of T_I and T_E which were not statistically significant. The effect of PDG, however, was still highly significant (Table 2).

The action of injected PDG in accelerating breathing was seen to survive vagotomy in the present study. This is in contradiction to our (and others) earlier findings in which the effects were virtually abolished. We believe that this difference results from the existence of a period of several minutes immediately after vagotomy, even after a vagotomized pattern of breathing has been established, when the rabbit is more or less insensitive to PDG. We can speculate that this insensitivity may be due to the trauma of vagal section, either the barrage of injury potentials synchronously produced in all fibres of the vagus, or the sudden cessation of vagal afferent activity, or both. This raises the question of whether other methods of cutting off afferent information in the vagi such as cold block and the application of direct current to the nerve may also produce a period of insensitivity. Other workers (Trenchard & Widdicombe, 1973) have reported that differential block of conduction by direct current results in a transient perturbation in pattern of breathing and this may include or precede a period of insensitivity of the type we have reported.

By selection of dose, intrapericardial local anaesthetic and by nerve section we endeavoured to exclude the somatic sites at which PDG may affect respiration, but still its effect persisted. This leads us to the suggestion that PDG may have a direct effect on the central nervous system. Against this hypothesis is the fact that the guanidinium moiety has a pK of 12·5 and it is known that strongly ionized agents such as quaternary amines do not rapidly cross the blood–brain barrier (Mayer, Melmon & Gilman, 1980). However, rapid transport can take place of specific substances and at specific sites in the brain (Bloom, 1980) and it may be that this is the case for PDG.

It is clear from the present study that the respiratory effects of PDG in the rabbit are not abolished by vagotomy, even when the drug is used in concentrations and with procedures designed to restrict its action to lung J receptors. Results obtained with PDG and attributed to the activation of J receptors in this species must therefore be reassessed.

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Upper respiratory tract resistance in dogs.

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Upper respiratory tract resistance and snoring in dogs

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ABSTRACT

We have developed a model to study snoring in supine, anaesthetised dogs (greyhounds). The trachea was cannulated both low in the neck and also 1-2 cm below the larynx, and the two cannulae were connected via a Fleisch pneumotachograph. Thus the dog breathed through the upper respiratory tract (URT). We recorded airflow, pharyngeal pressure via an oesophageal catheter, and tracheal pressure below the larynx, so that URT and oronasal resistances could be assessed from pressure/flow relationships. We also recorded the e.m.g. from genioglossus (airway dilator) muscle and the sound of snoring. In some dogs snoring was spontaneous, in others it was induced by gentle manual closing of the nares, and in others it could not be induced at all. Recording the same variables in the same dogs we measured URT resistances by passing air through the upper tracheal cannula and the URT from a gas cylinder via a rotameter while the dogs breathed through the lower tracheal cannula; flow/pressure curves were constructed. Insertion of 0.5-1 ml of Sonarex (0.2 g benzalkonium chloride, 2 g polysorbate 80, 2 g glycerol, 9 g NaCl, per litre) into the oropharynx promptly lowered URT and oropharyngeal resistances, increased genioglossus e.m.g. and decreased snoring when present. All results are statistically significant. 0.5 to 1 ml of 0.9 per cent saline had similar but smaller actions. Flow/pressure in the isolated URT measured 5-20 min before and after introduction of liquid into the oropharynx showed decreases in resistance due to Sonarex and increases in resistance due to saline. We conclude that Sonarex decreases snoring and URT resistance partly by a direct mechanical action and partly by reflex constriction of airway dilator muscles; saline has a smaller and more transient effect.

KEY WORDS

Snoring, genioglossus, dogs, oropharyngeal resistance, Sonarex.

INTRODUCTION

We have developed a model to study snoring, upper airway resistance and upper airway dilator muscle activity in anaesthetised dogs. We have used this model to test the effect of Sonarex (0.2 g benzalkonium chloride, 2 g polysorbate 80, 2 g glycerol and 9 g NaCl, per litre) introduced into the pharynx.

METHODS

Ten adult greyhounds of either sex were used, anaesthetised with intravenous pentobarbitone sodium (30 $mg.kg^{-1}$ initially) and placed supine. The caudal cervical trachea was cannulated low in the neck, and a similar cannula was inserted cranially pointing towards the larynx (Fig. 1). The cannulae were connected to a Fleisch pneumotachograph so that the animal breathed through the pneumotachograph and the URT. A plastic catheter was inserted through the oesophagus into the oropharynx, where its tip could be observed via the mouth. Sometimes a small balloon was tied over the tip of the catheter. This catheter was used for measuring pharyngeal pressure. Tracheal pressure was measured from the upper tracheal cannula. The records of airflow and the two pressures allowed measurement of upper airways resistance either including the larynx (from the tracheal cannula) or excluding the larynx (from the pharyngeal catheter). At intervals during recording the two nares were gently closed by hand so that the dog breathed through the mouth. This method allowed assessment of upper airways resistances during spontaneous breathing through the upper airways, from the pressure/flow relationships. The results could be divided into resistances in the various components of the URT.

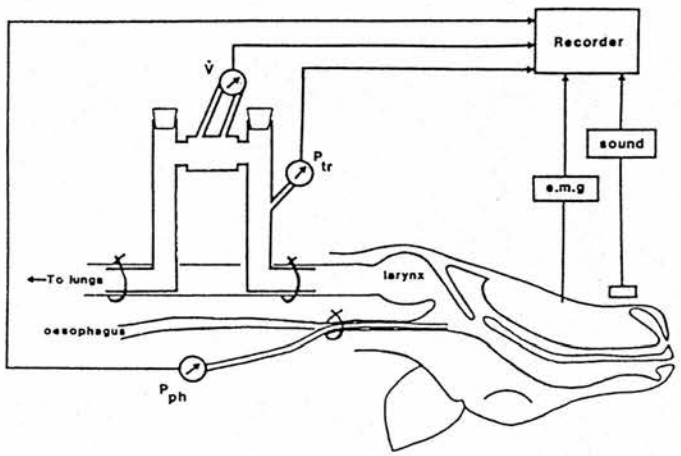

Fig. 1. Diagram of experimental arrangement

A modification of the method was to allow the dog to breathe through the lower tracheal cannula, and to have continuous airflow through the upper tracheal cannula and URT. Flow was measured with a rotameter and, by varying flow, flow/pressure curves could be constructed for the upper airways.

Additional measurements included an e.m.g. from wire-hook electrodes in the genioglossus muscle, the sound of snoring or upper respiratory airflow from a microphone taped to the neck of the dog, and systemic arterial blood pressure.

To test the effects of Sonarex the solution was injected through the catheter in the oropharynx in 0.5-1 ml volumes, during recording of respiratory variables. Controls with 0.9 per cent NaCl solution were also performed and randomised with tests of Sonarex. At least 2 h were allowed between injections of liquids.

All variables were recorded on magnetic tape and on a chart recorder. The records of genioglossus e.m.g. and snoring were later integrated using half-way rectification and a moving average integrator with a time constant usually 200 ms. Peak changes in e.m.g., sound and upper airway conductances are reported. Conductance is used rather than resistance (its reciprocal) because complete closure of the upper airways would lead to infinite resistance and this would invalidate averaging of results.

RESULTS

Administration of Sonarex into the pharynx while the dog was breathing through the URT caused a prompt increase in upper airway conductance. This varied with the respiratory phase since the upper airway dilates during inspiration because of contraction of the dilator muscles. When the nose was open resistance measured from the trachea increased conductance by +48 ± 18.2 per cent in expiration, and by +18 ± 7.2 per cent in inspiration ($p < 0.05$, $n = 15$). When the nose was closed corresponding increases in conductance were +37 ± 22.1 and +24 ± 8.6 per cent ($n = 13$). Administration of saline into the pharynx caused variable effects, although there was often an increase in upper airway conductance usually smaller than that seen with Sonarex.

Analysis of the pressure/flow curves of the upper airways show that before Sonarex the curves were remarkably alinear, presumably due to sudden changes in upper airway geometry. After Sonarex the curves were not only more smooth in appearance but also the upper airway resistance estimated from the curves was consistently reduced except at very high and very low flow rates. By contrast, when saline was administered into the oropharynx the pressure/flow curves were usually displaced upwards, i.e. indicating a higher resistance to airflow.

Analysis of the integrated e.m.g. from genioglossus muscle showed that administration of Sonarex increased the activity during inspiratory phases by +55.2 ± 15.4 per cent ($p < 0.01$, $n = 14$). By contrast, saline had a far smaller effect (+17.0 ± 6.0 per cent, $p < 0.05$, $n = 5$). Analysis of the integrated records of the sound of snoring showed that this was significantly reduced by Sonarex (-19.4 ± 8.0 per cent, $p < 0.05$, $n = 10$). In three experiments saline had inconsistent effects on the sound of snoring.

DISCUSSION

Our results show that both saline and Sonarex administered into the oropharynx decrease the sound of snoring and the resistances of the URT, and increase genioglossus muscle activity. However the results with saline are smaller and usually far less consistent that those with Sonarex.

By contrast, when pressure/flow curves were obtained for the URT, Sonarex consistently produced far smoother pressure/flow relationships and a decrease in upper airway resistance whereas saline, if anything, increased resistance. The difference between the results described in the previous paragraph and those here may be that the former give the immediate response to agents in the pharynx whereas the pressure/flow curves were obtained at a considerable interval (5-20 min) before and after the administration of Sonarex or saline. In other words Sonarex seems

to have a maintained action in increasing upper airways conductance whereas the effect of saline is very brief.

The mode of action of Sonarex has not been established, but the fact that genioglossus usually contracted more forcibly suggests that there may be reflex activation of this muscle and stimulation by the injection of fluids into the pharynx. Another possibility is that the adhesiveness of the oropharyngeal walls may be lessened by agents such as Sonarex or saline, although this in itself should not increase genioglossus activity. Sonarex contains four constituents as well as water, and our results suggest that sodium chloride and water are not the most active of these. Which of the other three is or are the most important in this respect requires further experiments.

RESUME

Nous avons developpé une methode pour étudier le ronflement sur des chiens (levriers) anesthésiés et couchés sur les dos. La trachée a été canulée bas dans le cou et aussi 1-2 cm au-dessous du larynx, et les deux canules ont été jointes par un pneumotachographe (Fleisch). Le chien donc respirait par les aériennes supérieures. Nous avons enregistré le débit aérien, la pression pharyngienne par un cathéter oesophagien, et la pression trachéale au-dessous du larynx pour dériver les résistances des aériennes supérieures et nasobuccales des relations débit/pression. Nous avons aussi enregistré l'e.m.g. du muscle genioglossus (dilatateur des aériennes) et le son du ronflement. Quelques chiens ont présenté un ronflement spontané, des autres ont ronflé quand leurs naseaux ont été fermées gentilement avec le main, et les autres n'ont pas ronflé n importe quelle leur condition. Nous avons aussi enregistré les mêmes variables dans les mêmes chiens, et nous avons measuré les résistances des aériennes superieures et nasobuccales; nous avons introduit de l'air dans la canule trachéale craniale et l'aérienne supérieure à l'aide d'un cylindre de gaz et un rotametre, lorsque les chiens respiraient par la canule trachéale inférieure. Nous avons donc derivé les curbes débit/pression. L'injection de 0.5-1 ml de Sonarex (0.2 g benzalkonium chloride, 2 g polysorbate 80, 2 g glycerol et 9 g NaCl, per litre) dans l'oropharynx a réduit rapidement les résistances des aériennes supérieures, a augmenté l'e.m.g. du genioglossus et a réduit le ronflement quand il se presentait. Tous les résultants étaient significatifs du point de vue de la statistique. 0.5-1 ml de saline 0.9 per cent ont montré des réponses similaires mais plus petites. Les curbes débit/pression des aériennes supérieures isolées, measurées 5-20 min avant et après l'injection du liquide dans l'oropharynx, ont montré une baisse de la résistance pour l'action du Sonarex et une augmentation de la résistance pour l'action de saline. Notre conclusion est que le Sonarex baisse le ronflement et la résistance des aériennes supérieures en partie par une action méchanique et directe, et en partie par la contraction reflexe des muscles dilatateurs des aériennes supérieures. La saline a un effet moins marqué et plus transitoire.

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480 Effect of "Sonarex" on Upper Airway Tone.

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Sleep apnoea in adults may arise from anatomical or physiological factors predisposing the airways to obstruction.

Lack of tone in the muscles of the pharyngeal region would allow collapse under the negative pressures of inspiration. A preparation (Sonarex) insufflated into the nose at night is reported to relieve snoring, which is associated with sleep apnoea. We have tested this preparation in the isolated in-vivo upper airways of anaesthetised rabbits.

Pressure in the pharynx was measured while flow was slowly increased by a vacuum pump drawing air through a tube directed rostrally through the vocal folds of the larynx.

A point was reached where increasing the driving pressure no longer increased flow. Driving pressure was then slowly reduced to zero. The procedure was repeated after 0.1ml of saline or Sonarex was injected into the pharyngeal lumen. Airflow was found to be significantly modulated by the airways above the larynx. There was hysteresis between increasing and decreasing pressure/flow relationships. A maximum flow was reached which could not be exceeded by increasing driving pressure along the airway. Sonarex or saline displaced the pressure/flow loops in different directions.

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Upper airways resistance and snoring in anaesthetized dogs

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Upper airways resistance and snoring in anaesthetized dogs. J.G. Widdicombe, A. Davies.

ABSTRACT: We have measured upper airways resistance from the trachea and from the pharynx to the atmosphere, EMG of genioglossus muscle, and the sound of snoring, in anaesthetized greyhounds breathing spontaneously through the upper airways. Using extra-corporeally produced continuous flow we determined flow/pressure curves for the upper airways in an expiratory direction and analysed them in terms of resistances from the trachea and from the pharynx. Resistances and other variables were determined with the nose open and the nostrils blocked. About one-third of the dogs snored spontaneously and most of the remainder did so when the nose was blocked. During snoring with nasal blockage the upper airways resistance increased considerably, and the sound of snoring and genioglossus EMG were also enhanced. The results show that the anaesthetized greyhound is a suitable model for studying snoring with simultaneous measurements of upper airways resistance and the activity of pharyngeal dilator muscles.

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Keywords: Genioglossus; pharynx; snoring; upper airways resistance.

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In man, snoring is recognized as a potential medical problem, possibly leading to obstructive sleep apnoea and progressive cardiovascular disease such as hypertension [1–4]. In addition it can be a social problem of serious proportions which are sometimes neglected because of humorous conotations. Medical and surgical treatments of snoring are developing rapidly, but some of the surgical interventions, which are undoubtedly necessary in severe cases, are formidable and most medical treatments require further research to test their efficacy.

Physiological studies in man have established that during snoring the pharyngeal cross-sectional area is reduced. If the pharyngeal dilator muscles contract less forcibly, intraluminal pressures that close the pharynx need to be less negative than usual to cause pharyngeal collapse during the inspiratory phase [5–7]. This results in increased upper airways resistance and vibration of soft tissue around the oropharynx which leads to the typical noise of snoring, as well as greater intrathoracic negative pressures in inspiration to overcome the increased oropharyngeal resistance. Snoring is normally prevented by contraction of the inspiratory dilator muscles of the oropharynx, which maintain upper airway patency [8, 9]. The physiological control of these muscles has recently been studied in man and experimental animals [8–12]. Another possibility, which has been little tested, is that the presence or absence of upper airway secretions and their chemical nature may lead to increased adhesiveness of the soft tissues of the upper airways [13, 14]. Furthermore, vibration of secretions in the upper airways may contribute to the sound of snoring.

To understand the mechanism of snoring it would be useful to have an animal model. Dog owners know well that members of the species snore, especially if the dog is old and fat: these last features may suggest a comparison with snoring in man [1]. Bradycephalic dogs have upper airways obstruction [15]. The sleeping bulldog snores, and sleep apnoeas and hypoxic episodes have been studied [16]; however, availability and expense may limit the use of the bulldog in snoring studies. We have, therefore, studied anaesthetized greyhounds breathing through the upper respiratory tract to see whether or not snoring occurred or could be induced, and the relationship between snoring and upper airway mechanics and genioglossus (airway dilator muscle) activity. A subsequent paper [17] describes changes in the measured variables that follow introduction of surface-active agents into the oropharynx.

Methods

Ten adult greyhounds of either sex were used (body weight range 26–32 kg). They were anaesthetized with intravenous sodium pentobarbitone (30 mg·kg^{-1} initially) and placed in a supine position. Blood pressure was recorded through a catheter in a femoral artery with a strain-gauge manometer. A femoral vein was catheterized for injection of supplementary doses of anaesthetic. An L-shaped plastic cannula was tied into the cervical trachea as caudal as possible in the neck, and a similar cannula was inserted pointing cranially below the larynx (fig. 1.); this was positioned with its tip 2–3 cartilage

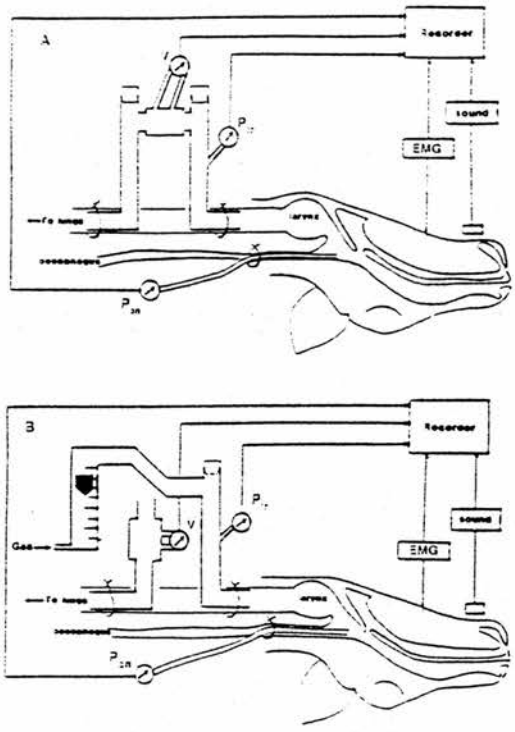

Fig. 1. – Diagram of experimental arrangements. (A) with the dog breathing through the upper airways; (B) with controlled flow through the upper airways to determine flow/pressure relationships. For details see text.

rings below the cricoid cartilage. Care was taken to avoid damage to the recurrent and pararecurrent laryngeal nerves. A plastic catheter, internal diameter 2 mm, was passed via the midcervical oesophagus into the oropharynx, where its tip could be observed via the mouth. It was tied in place by a snare around the oesophagus, sparing the laryngeal nerves.

The electromyogram (EMG) of the genioglossus muscle was recorded via two fine-wire hook electrodes, positioned with their tips about 5 mm apart. The sound of snoring was recorded with a microphone either attached to a canine tooth of the upper jaw or mounted about 2–3 cm away from the side of the mouth. Upper airways pressures were recorded through the air-filled pharyngeal catheter and through the side of the cranial tracheal cannula by strain-gauge manometers (Gould). Airflow was recorded by a Fleisch pneumotachograph. Airflow, tracheal and pharyngeal pressures, EMG and sound were all recorded on magnetic tape (Racal) and on recording paper (Gould).

Two experimental procedures were performed. In the first, the pneumotachograph connected the two tracheal cannulae so that the dog breathed through its upper respiratory tract (fig. 1A). In this condition most dogs did not snore, but snoring could be induced by closing the nostrils with gentle manual pressure. To prevent the accumulation of respiratory effects of nasal obstruction, this was performed for 1–2 breathing cycles about once every minute during experimental runs. On a few occasions the nostril on one side only was closed to induce snoring.

In the second procedure the dog breathed through the caudal tracheal cannula and the pneumotachograph (fig. 1B). The cranial tracheal cannula was connected to a rotameter and a compressed air cylinder, and air was blown through the upper repiratory tract in steps of 1l·min^{-1} from 0–60 l·min^{-1}. Each step was held for about 20 s. At any constant flow rate, pressure varied with respiratory phase and peak inspiratory and expiratory pressures were measured. This allowed the preparation of flow/pressure curves for the upper respiratory tract for inspiratory and expiratory phases during constant expiratory flow. The procedure was carried out first with the nose open and then with the nose closed.

Analysis of results

Changes in the intensity of EMG and sound could usually be heard and seen clearly on the chart record. These were later quantitated by integration from the magnetic tape using half-wave recufication and a moving average integrator with a time constant of 200 ms [17]. Upper airways resistances were determined from the tracheal and pharyngeal pressure records and the airflow. The ratios of peak pressures to airflows were used to calculate resistances, which therefore corresponded to those at peak inspiratory and expiratory airflows.

The flow/pressure curves were graphically plotted. Their alinearity complicates the use of a single value for resistance (see Discussion). The sound recorded during production of a flow/pressure relationship was recorded as described above. Values given are mean±SEM.

Results

In three of the ten dogs breathing through their upper respiratory tracts, snoring was present from the beginning of the experiment; but in six dogs snoring occurred only when the nostrils were closed (fig. 2). The intensity depended on the position of the tongue. Pulling the tongue forward seemed to lessen snoring, so the tongue was left relaxed in the partially open mouth. Any tongue and jaw position was maintained throughout the experimental procedures. In one dog snoring never occurred, even with the nostrils closed, whatever the tongue position. In this dog lateral external pressure on the pharyngeal wall or extreme flexion of the neck induced snoring.

Spontaneous breathing through upper airways

Table 1 gives control values for total upper airways resistance from trachea and pharynx to the atmosphere, and "laryngeal resistance" in the inspiratory and expiratory phases, with the nose open and the nose closed. Laryngeal resistance was derived by subtraction of resistance recorded from the pharynx from that measured

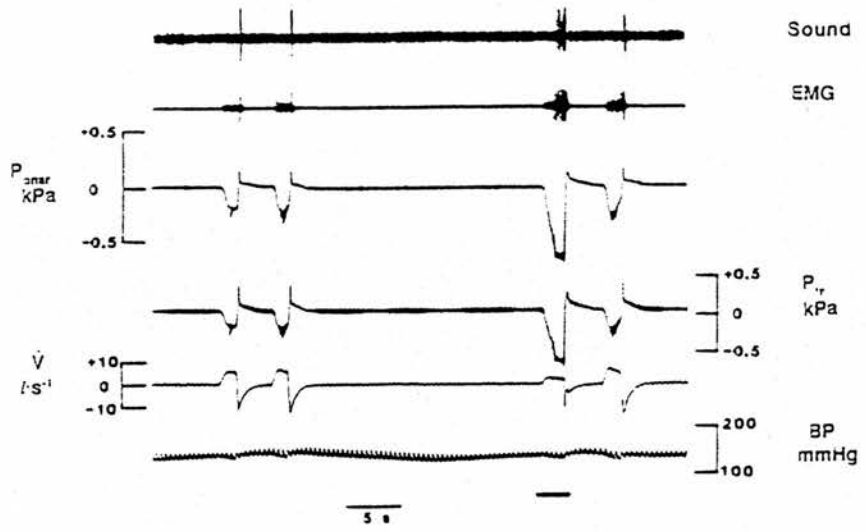

Fig. 2. – Record of physiological variables and sound of snoring. Traces from above down: sound, genioglossus EMG, pharyngeal pressure (Ppn), tracheal pressure (Ptr), tracheal airflow (V) and blood pressure (BP). The dog was breathing spontaneously through its upper airway (as in fig. 1A). On the left-hand side of the record, two spontaneous breaths occur with EMG activity during inspiration, and with a sharp peak of EMG activity and of sound at the beginning of expiration. On the right-hand side, during the signal bar, the nostrils were closed for one breath, resulting in increased upper airway pressures and decreased airflow. At the same time inspiratory EMG was greatly enhanced, and the sound appeared strongly during inspiration.

Table 1. – Values of upper airway resistances measured during spontaneous airflow

Condition	Respiratory phase	Resistances with pressure measurements from: Trachea $kPa \cdot l^{-1} \cdot s$	Pharynx $kPa \cdot l^{-1} \cdot s$	Trachea-pharynx $kPa \cdot l^{-1} \cdot s$
Nose open	Inspiration	0.31±0.17	0.26±0.14	0.052±0.056
	Expiration	0.28±0.19	0.24±0.16	0.045±0.037
Nose closed	Inspiration	5.21±5.03	4.46±4.15	0.750±0.276
	Expiration	1.41±1.58	1.24±1.53	0.170±0.306

Means±SEM, n=8–10.

from the trachea. With the nose open, inspiratory and expiratory resistances were similar and laryngeal resistance was about one-sixth that of the total upper airways. When the nose was closed inspiratory resistances from the trachea and pharynx became 3–4 times larger than expiratory; the inspiratory laryngeal resistance became a smaller proportion (7%) of the total upper airways resistance. Laryngeal resistances were 4–15 times higher with nose closed compared to nose open, presumably because the segment of the airway between tracheal and pharyngeal pressure points included some collapsible oropharynx. When the nose was closed there was usually an increased sound of snoring, sometimes in both inspiratory and expiratory phases, and increased activity in the EMG of genioglossus in inspiration (fig. 2) with the nose open genioglossus EMG was sometimes active in the expiratory phase also.

Flow/pressure relationships

Figure 3 shows curves relating pressure to flow measured simultaneously from the trachea and from the pharynx in the inspiratory and expiratory phases with the nose closed (A and B), and with the nose open (C and D) in one experiment. A common feature of the relationships is that the flow/pressure curves are highly irregular in shape, usually showing a pronounced decrease in pressure (and therefore resistance) at the low or middle flow rates. As will be considered in the Discussion, this may be due to abrupt changes in the position of the epiglottus and of the soft palate. Figure 4 shows an experimental record of flow/pressure determination, selected partly because it illustrates marked irregularity of pressures and sound.

Table 2. – Values of upper airways resistances measured during continuous airflow

Condition	Respiratory phase	Resistances with pressure measurements from: Trachea kPa·l⁻¹·s	Pharynx kPa·l⁻¹·s	Trachea-Pharynx kPa·l⁻¹·s
Nose open	Inspiration	0.20±0.04	0.13±0.03	0.055±0.021
	Expiration	0.22±0.02	0.18±0.02	0.044±0.011
Nose closed	Inspiration	0.42±0.10	0.34±0.10	0.047±0.019
	Expiration	0.44±0.05	0.38±0.04	0.086±0.016

Means±SEM, n=7–10. Values correspond to a flow rate of 1 $l \cdot s^{-1}$ (60 $l \cdot min^{-1}$).

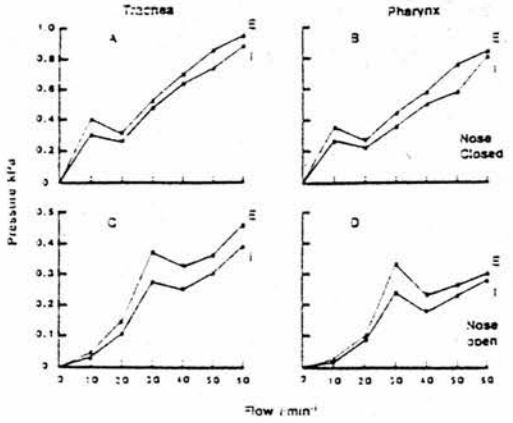

Fig. 3. – Flow/pressure curves drawn from a single experiment, with flows on the abscissa and pressures on the ordinate. On the left (A, C) pressures from the trachea, on the right (B, D) pressures from the pharynx. The upper records (A, B) show relationships with the nose closed, and the lower records (C, D) with the nose open. For each condition there are two curves, the upper one corresponding to pressures during expiration (E) and the lower one to pressures during inspiration (I). All curves show marked irregularities in the early (nose closed) or middle (nose open) parts of the ranges.

During measurement of flow/pressure relationships, genioglossus activity was usually absent or weak (fig. 4), but sometimes increased at higher flow rates or during irregularities of pressure patterns. Mechanical stimulation of the upper airways is known to cause reflex contraction of the pharyngeal dilator muscles [9, 11]. The sound of airflow appeared at the higher flow rates (fig. 4), especially when the nose was closed.

It will be clear from figure 3 that the irregularity of the flow/pressure curves does not allow the upper airways to be defined by a single resistance using this method. However table 2 presents mean results for "resistances" at the maximum flow rate of 60 $l \cdot min^{-1}$, assuming linearity of the resistance relationship (*i.e.* measured pressure divided by 60 $l \cdot min^{-1}$). Resistances were lower than those during spontaneous breathing through the upper airways. When the nose was closed resistances measured from the trachea and pharynx were approximately doubled; however, they were considerably lower than those measured during spontaneous airflow. The lower resistances measured during continuous compared to spontaneous airflow were presumably due to the fact that airflow at 60·min^{-1} in the expiratory direction would distend the oropharynx; this would prevent the collapse of this region

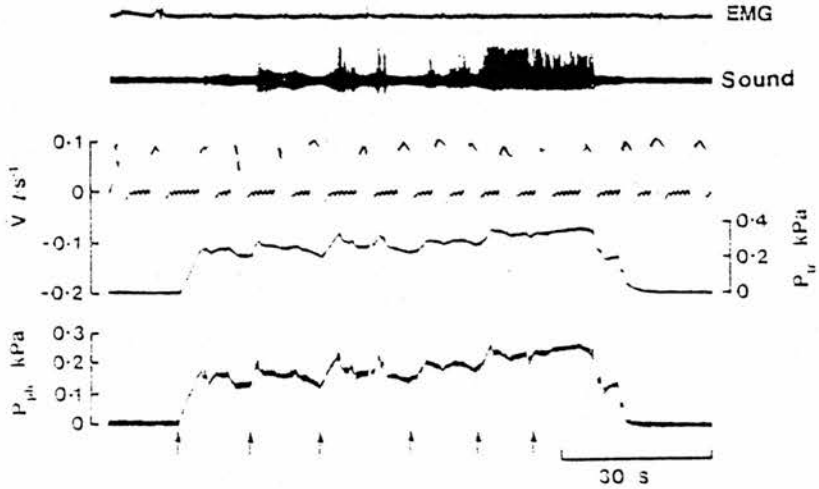

Fig. 4. – Record showing traces from which flow/pressure relationships were derived. Traces from above down: genioglossus EMG, sound, airflow (V), tracheal pressure (Ptr), pharyngeal pressure (Pph), and blood pressure (BP). Flow was increased in steps of 10·min^{-1} up to 60·min^{-1} at the arrows. The pressure records show marked irregularity especially at the lower flow rates. At the higher flow rates sound appeared and increased. Genioglossus EMG was absent apart from weak inspiratory bursts of activity. During this record the nose was closed.

with high resistances especially during inspiration seen with spontaneous breathing when the nose was closed.

Discussion

The control of upper airways patency and resistance has been intensively studied in the last few years. This is partly because of the interest in the physiological mechanisms acting on upper airways muscles [8, 9, 11, 12] and also because of the importance of both obstructive apnoea and snoring; the latter may be a possible preclinical condition leading to more serious states [1–4]. Inevitably, most of the physiological studies of upper airways muscles have been with experimental animals, and most of the studies of obstructive sleep apnoea have been with man. For this reason there is an advantage in an animal model of snoring in which mechanisms of control can be analysed. Although there have been a number of studies on breathing in sleeping animals and on mechanisms of pharyngeal obstruction in anaesthetized animals [13, 18, 19], these seem to have been related to snoring for the bulldog only [16]. For this reason the model we have developed may be of value.

Snoring in anaesthetized greyhounds is rather similar to that in man, in that about one-third of the dogs snore spontaneously and most of the remainder do so when the nose is blocked [1, 20]. Occasional dogs will not snore under any circumstances apart from external obstruction of the airways or severe neck flexion. In the dog, gender seems to make no difference whereas in man snoring is more common in males [1]. We cannot say whether obesity is a factor influencing upper airways resistance and snoring since all the greyhounds we used were lean, presumably because of their lifestyle. The dogs were anaesthetized, whereas snoring humans are usually studied unanaesthetized but asleep; the airway control mechanisms in our study may therefore be quantitatively different from those in man.

The physics of upper airways "resistance" is formidable [8, 19]. Not only do maintained flow/pressure relationships show great alinearity (fig. 3) but, with spontaneous breathing through the upper airways, the pressure and flow records also have marked oscillations (fig. 2); this of course is to be expected because, almost by definition, snoring implies that the soft tissue elements of the upper airways are rapidly collapsing and distending, thereby imposing dramatic changes in spontaneous resistance as high as infinity when there is complete obstruction. For this reason, for spontaneous breathing we have only expressed our results as the peak inspiratory and expiratory resistances. Attempts to obtain meaningful values from pressure/flow loops displayed during spontaneous breathing through the upper airways were unsuccessful.

An additional problem is that pharyngeal pressure during spontaneous breathing is very low, especially when the nose is open. Also the pharyngeal catheter can easily become blocked with mucus. Hence N-values for pharyngeal pressures were sometimes smaller than those for tracheal. However, the results obtained with pharyngeal pressure were generally paralleled by those with pressures from the trachea below the larynx. The translaryngeal pressure was normally extremely low and may have included a component of the oropharynx due to the position of the pharyngeal catheter; there was no clear inspiratory decrease in "laryngeal" resistance, possibly because any decrease was counterbalanced by an increase in resistance due to collapse of this oropharyngeal segment.

The flow/pressure curves derived from continuous flow through the upper airways were highly irregular in shape (fig. 3), possibly because of sudden changes in the position of the epiglottis and the soft palate as flow was increased through the upper airways. In experiments in which resistances were not measured, these rearrangements could be seen by looking through the mouth. With this method it is possible to obtain resistances with the flow only in the expiratory direction; if negative pressure is applied to the upper trachea to draw air through the upper airways in the inspiratory direction, there is complete collapse of the oropharynx and larynx in the expiratory phase, with a ball-valve effect.

Tables 1 and 2 show that upper airways resistances are extremely variable, as indicated by the standard errors of the means. This is not surprising in view of the great variability of the geometry of the upper airways in natural and experimental conditions.

The sound of snoring can occur in both inspiratory and expiratory phases, although it is usually louder in the former (fig. 2). Dictionary definitions do not state that snoring has to be an inspiratory noise. The aerodynamics of continuous flow in an expiratory direction through the upper airways, producing a sound due to the air movement, is clearly very different from spontaneous flow through upper airways and natural snoring. Nonetheless, the continuous flow method has the advantage that flow rates are carefully controlled and resistance curves, although very alinear, can be plotted. Therapeutic or surgical methods which might change upper airways function could be tested by either or both methods.

In a subsequent paper [17] we describe the effect of a mixture of surface-active agents on the variables described here, and show that the model we have developed is suitable for studying the therapy of upper airways obstruction and snoring.

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RÉSUMÉ: Nous avons mesuré la résistance des voies aériennes supérieures entre la trachée et le pharynx d'une part et l'atmosphère d'autre part ainsi que l'électromyogramme du muscle génioglosse et les bruits de ronflement chez des lévriers anesthésiés. Nous avons également déterminé les courbes débit/pression pour les voies aériennes supérieures, en utilisant un débit continu, produit de façon extra-corporelle dans une direction expiratoire. Nous avons analysé l'ensemble en terme de résistance au niveau de la trachée et du pharynx. Les résistances et les autres variables ont été mesurées avec le nez ouvert et avec les narines bloquées. Environ 1/3 des chiens ronflaient spontanément et la plupart des autres le faisait lorsque le nez est bloqué. Les mesures de résistance ont montré qu'au cours du blocage nasal, avec ronflements, les résistances des voies aériennes supérieures augmentent considérablement et que le bruit de ronflement ainsi que l'électromyogramme du génioglosse sont stimulés. Les résultats montrent que le lévrier anesthésié est un modèle adéquat pour l'étude du ronflement avec des mesures simultanées des résistances des voies aériennes supérieures et de l'activité des muscles dilatateurs du pharynx.

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The effects of a mixture of surface-active agents (Sonarex) on upper airways resistance and snoring in anaesthetized dogs

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The effects of a mixture of surface-active agents (Sonarex) on upper airways resistance and snoring in anaesthetized dogs. J.G. Widdicombe, A. Davies
ABSTRACT: We measured upper airways resistance from the trachea and from the pharynx to the atmosphere, EMG of the genioglossus muscle and the sound of snoring, in anaesthetized greyhounds, breathing spontaneously through the upper airways. Using extra-corporeally produced continuous flow we determined flow/pressure curves for the upper airways and resistances from the trachea and from the pharynx. We tested the effects of 0.9% saline and of Sonarex (a proprietary mixture containing sodium chloride, glycerol, polysorbate 80 and benzalkonium chloride). Both saline and Sonarex decreased upper airways resistance, but the latter did so more consistently. With Sonarex, genioglossus activity increased and the sound of snoring decreased. Flow/pressure curves 5–20 min after Sonarex showed a decrease in upper airways resistance and a smoother curve, whereas those with saline showed an increase in resistance. The sound produced by continuous flow through the upper airways was decreased by Sonarex but increased by saline. Thus, both Sonarex and saline decrease upper airways resistance, but Sonarex also reduces the sound of snoring and the resistance and sound of continuous airflow through the upper airways.
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Keywords: Genioglossus; snoring; Sonarex; upper airways resistance.

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We have described a method for measuring upper airways resistance, genioglossus electromyogram (EMG) and the sound of snoring in anaesthetized greyhounds [1]. The present paper describes the effects of saline and of a proprietary treatment for snoring (Sonarex) on these variables. The liquids were inserted into the oropharynx. Some of the results have been presented as an abstract [2].

Methods

Twelve adult greyhounds were used. Nine were the same dogs as described in a previous paper [1]. In brief, they were anaesthetized with pentobarbitone sodium (30 mg·kg^{-1} initially), and blood pressure and tracheal airflow were measured. The latter was obtained from a cannula inserted in the lower cervical trachea. A similar cannula was placed in the upper cervical trachea, pointing cranially. A plastic catheter, internal diameter 2 mm, was passed *via* the mid-cervical oesophagus into the oropharynx. This was used for pressure recording and for injection of liquids. The electromyogram of the genioglossus muscle was recorded *via* hook electrodes, and the sound of snoring was recorded from a microphone either attached to a canine tooth or mounted 2–3 cm away from the side of the mouth.

Two experimental procedures were performed. In the first the pneumotachograph connected the two tracheal cannulae so that the dog breathed through its upper respiratory tract. Airflow, tracheal and pharyngeal pressures, EMG and sound were all recorded on magnetic tape (Racal) and on recording paper (Gould).

In this condition some dogs did not snore but snoring could be induced by closing the nostrils with gentle manual pressure. To prevent the respiratory effects of nasal obstruction, this was performed for 1–2 breathing cycles about once every minute during experimental runs. On a few occasions the nostril on one side only was closed to induce snoring.

With the second procedure the dog breathed through the caudal tracheal cannula and the pneumotachograph. The cranial tracheal cannula was connected to a rotameter and a compressed air cylinder, and air was blown through the upper respiratory tract in steps of 10 l·min^{-1} from 0–60 l·min^{-1}. Each step was held for about 20 s. At any constant flow rate, pressure varied with respiratory phase, and peak expiratory and inspiratory pressures were measured. This allowed the preparation of flow/pressure curves for the upper respiratory tract for inspiratory and expiratory phases. The procedure was carried out first with the nose open and then with the nose closed.

When the dog was either snoring spontaneously or because its nostrils were closed, a control record was made for several minutes, if necessary closing the nostrils for

one or two breaths every minute. The two tracheal cannulae were then disconnected and flow/pressure curves of the upper respiratory tract were determined first with the nose open and then with the nose closed. Duplicates of each curve were made. The two tracheal cannulae were then reconnected *via* the pneumotachograph so that the dog again breathed through its upper respiratory tract.

After several minutes of recording, if necessary with closure of the nose, 0.5 or 1.0 ml of either Sonarex (see below) or saline was introduced through the pharyngeal catheter, which could not be used for pressure recording for about 30 s during this injection. The solution was blown in with air, and pressure recording was restored promptly. After recording variables with breathing through the upper respiratory tract for about a further 5 min, the two tracheal cannulae were again separated and flow/pressure curves of the upper respiratory tract were determined with nose open and nose closed, in duplicate. The whole procedure took about 20 min. Supplemental doses of anaesthesia were given at intervals of about 60 min, and never during a procedure as described above.

Saline was made up as 9 $g \cdot l^{-1}$ sodium chloride. Sonarex is a mixture of sodium chloride (9 g), glycerol (85%, 3 g), polysorbate 80 (Tween 80, 2 g) and benzalkonium chloride (0.2 g), all quantities per litre of water: it was provided by Anasco GmbH. The choice of solution for the initial test in each dog was randomized, and the other solution was used subsequently. Usually at least 2 h were allowed between injections of liquids. In some experiments the oropharynx was cleaned out with gauze swabs between introductions of liquids. In some dogs a third injection of liquid was made about 2 h after the second. Results were analysed by analysis of variance and Student's paired t-test.

Results

Figure 1 shows an example of the action of Sonarex on airflow, upper airway pressures and genioglossus EMG. Before Sonarex (A), the genioglossus contracted only in the inspiratory phase and there were rapid oscillations in inspiratory flow and pressure corresponding to snoring. Immediately after the application of Sonarex there was an increase in genioglossus activity in both inspiratory and expiratory phases (B). Within 30 s the rapid oscillations in flow and pressure had decreased (C), the pharyngeal and tracheal pressure swings were smaller especially in the inspiratory phase and flow was, if anything, greater. In other words there were decreases in upper airway resistances measured from the two sites concomitant with increased contraction of genioglossus.

Table 1 summarizes the percentage changes in upper airways resistance, measured from the trachea and pharynx, on insertion of saline and Sonarex into the oropharynx. There was considerable variation in response, as can be seen from the size of the standard errors of the means. However, both saline and Sonarex usually decreased upper airways resistances, especially those measured from the pharynx, whether the nose was open or closed.

Statistically Sonarex significantly decreased resistances in the inspiratory phase in all but one condition. Because of the high variance of control values, population means for Sonarex were no different from those for saline except for pharyngeal resistance in inspiration with the nose closed. However, with paired values, the responses to Sonarex were significantly greater than those to saline in half the conditions assessed (table 1).

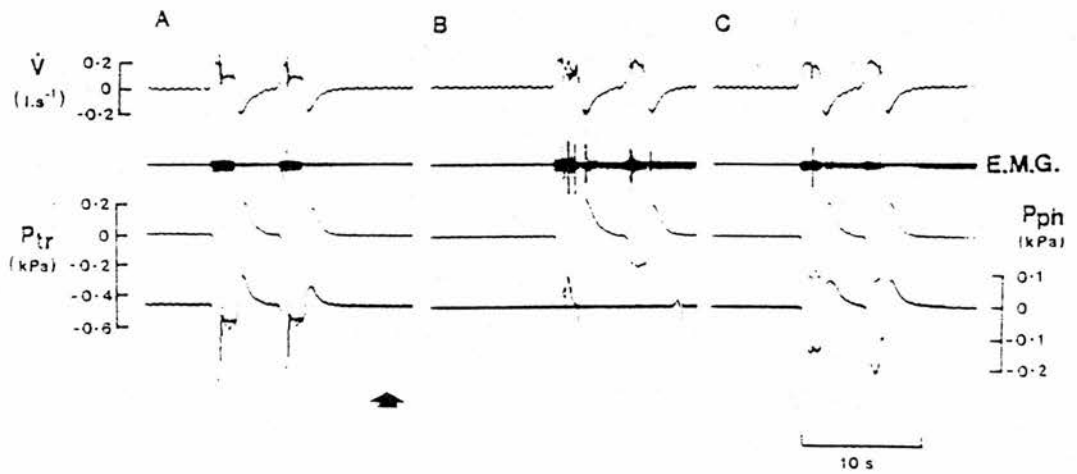

Fig. 1. – Responses to addition of 0.5 ml of Sonarex into the pharynx. Traces from above down: flow ($\dot{V}$), EMG from genioglossus, tracheal pressure (Ptr) and pharyngeal pressure (Ppn). (A) shows two control breaths. The pharyngeal pressure catheter was then disconnected and 0.5 ml of Sonarex was injected into the pharynx just before recording (B). (B) shows two breaths after addition of Sonarex. (C) shows two breaths 30 s later when the pharyngeal pressure recording catheter had been reconnected. Sonarex increased the genioglossus EMG in inspiratory and expiratory phases, decreased pharyngeal pressure swings, increased airflow and lessened airflow oscillations.

Table 1. – Changes in upper airways resistance on addition of saline or Sonarex to the oropharynx.

			Expiration		Inspiration	
Pressure	Nose	n	Control kPa·l⁻¹·s	Change %	Control kPa·l⁻¹·s	Change %
Saline						
Tracheal	Open	11	0.69±0.16	-8±3.3*	0.59±0.11	-1±3.4
	Closed	11	3.48±3.72	+1±8.4	8.58±7.12	-16±6.1*
Pharyngeal	Open	9	0.38±0.10	-14±7.5*	0.35±0.10	-15±10.6
	Closed	9	3.21±2.86	-8±7.5	7.49±6.53	-4±12.2
Sonarex						
Tracheal	Open	15	0.66±0.14	-19±9.7**+	1.02±0.22	-11±4.9*+
	Closed	13	1.32±0.34	-17±7.0**+	5.20±1.15	-28±8.9**
Pharyngeal	Open	14	0.53±0.16	-9±3.5**	0.62±0.15	-28±4.7**
	Closed	12	1.28±0.26	-13±12.1	3.99±1.15	-29±6.9**+

Values are means±SEM. * p<0.05; ** p<0.01 for change compared to control; + p<0.05 for Sonarex compared to saline, paired values.

Analysis of the results in terms of translaryngeal pressure, obtained by subtracting pharyngeal from tracheal pressures and calculating laryngeal resistance, showed that neither saline nor Sonarex had a significant effect on laryngeal resistance. Because of the relatively small sizes of laryngeal resistance [1] it was difficult to measure changes in this variable accurately.

Integrated sound and integrated genioglossus EMG were measured before and immediately after introduction of saline or Sonarex into the pharynx (table 2). Sonarex significantly decreased sound by -19%, whereas saline increased sound by 11% (NS). Both saline and Sonarex significantly increased genioglossus EMG in the inspiratory phase and this was significantly larger for Sonarex compared to saline. In only one of fourteen tests did Sonarex fail to increase genioglossus EMG.

Figure 2 illustrates the results of injection of Sonarex into the pharynx on genioglossus EMG and its integral, and on pharyngeal pressure and airflow. Sonarex increased the activity of genioglossus in the inspiratory phase and tonic activity during expiration. Pressure swings in the pharynx were reduced although airflow values were maintained. Thus resistance measured from the pharynx was reduced.

Figure 3 shows curves relating pressure to flow measured simultaneously from the trachea and the pharynx with the nose open, in the expiratory phase, in one experiment. A common feature of the relationships is that before Sonarex the flow/pressure curves are highly irregular in shape, usually showing a pronounced decrease in pressure (resistance) at the middle flow rates (see [1] and Discussion).

To compare results between dogs we normalized the curves. The flow/pressure curves before Sonarex or saline were drawn with the maximum pressure at maximum flow (60 $l \cdot min^{-1}$) as 100%, and all the values at lower flows and after administration of Sonarex or saline are expressed as percentages of this value. The total number of pairs of curves is eight: pressures from pharynx and trachea, nose open and nose closed, inspiratory and expiratory phases. In figure 4 the effects of Sonarex (left) and saline (right) are shown for pharyngeal pressure with the nose closed in the expiratory phase. Sonarex displaced the curve downwards (*i.e.* reduced

Table 2. – Changes in integrated sound and integrated genioglossus EMG on addition of saline or Sonarex to the oropharynx.

		Saline		Sonarex	
Variable	Condition	n	Change %	n	Change %
Sound	Spontaneous flow, nose open	7	-11±19.0	10	-19±8.0*
	Continuous flow, nose open	5	-10±8.7	9	-65±9.2**
	Continuous flow, nose closed	5	-55±16.4*	7	-47±15.6*+
EMG	Spontaneous flow	8	-16±5.4**	14	-55±15.4**+

Means±SEMs. * p<0.05; **p<0.01 for change compared with zero effect; + p<0.05 for response to Sonarex compared with that to saline.

airflow resistance) and the curve was smoother. By contrast, saline (right) displaced the curve upwards whilst also making it smoother. The other seven conditions gave similar patterns: Sonarex displaced the flow/pressure curves downwards whereas saline displaced the curves upwards. These changes were especially conspicuous in the middle part of the curves. However, statistical significance was not always as clear as in figure 4, possibly because N-values are sometimes smaller.

Analysis of genioglossus EMG activity during flow/pressure curves is not presented because genioglossus activity was often weak or absent when the animals were not breathing through the upper respiratory tract, and because imposition of flow through the upper airways often reflexly increased genioglossus activity [1, 3–5]. With regard to sound, figure 5 shows the effect of Sonarex, with a time interval of about 20 min between records. In (A), determination of the flow/pressure

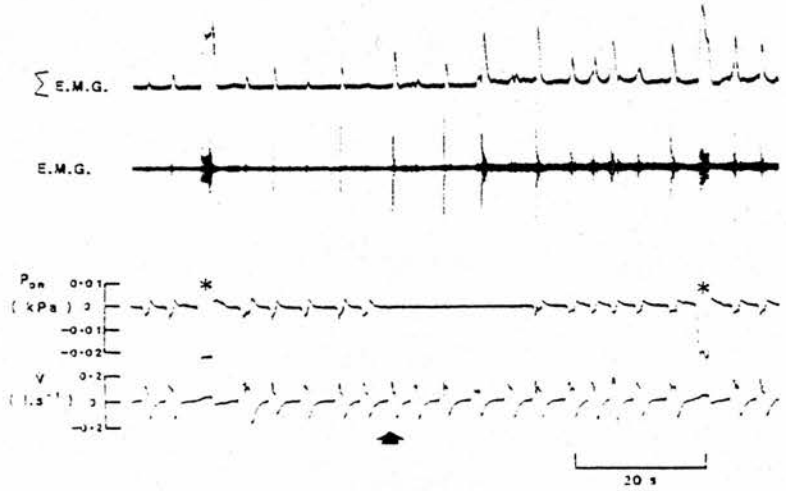

Fig. 2. – The effect of Sonarex on genioglossus EMG and its integral. Traces from above down: integrated EMG, genioglossus EMG, pharyngeal pressure (Ppn), and airflow (V). Sonarex was inserted into the pharynx at the arrow, while the pharyngeal pressure catheter was disconnected. It caused a prompt increase in genioglossus EMG and its integral and a reduction in the pharyngeal pressure oscillations when the pressure recorder was reconnected. The two breaths marked with asterisks correspond to closures of the nose.

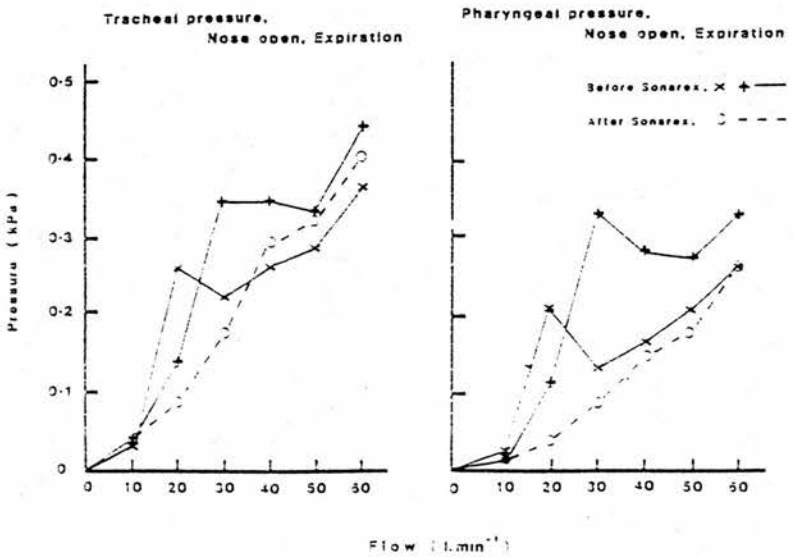

Fig. 3. – Flow/pressure curves drawn from a single experiment with the nose open and measuring pressures in the expiratory phase. On the left ordinate tracheal pressures are plotted and on the right ordinate pharyngeal pressures. Crosses correspond to control values and show marked irregularity of shapes. Open circles correspond to measurements 15 min after placing 0.5 ml of Sonarex into the pharynx. The curves are smoother and are displaced downwards especially for pharyngeal pressure and in the middle parts of the range.

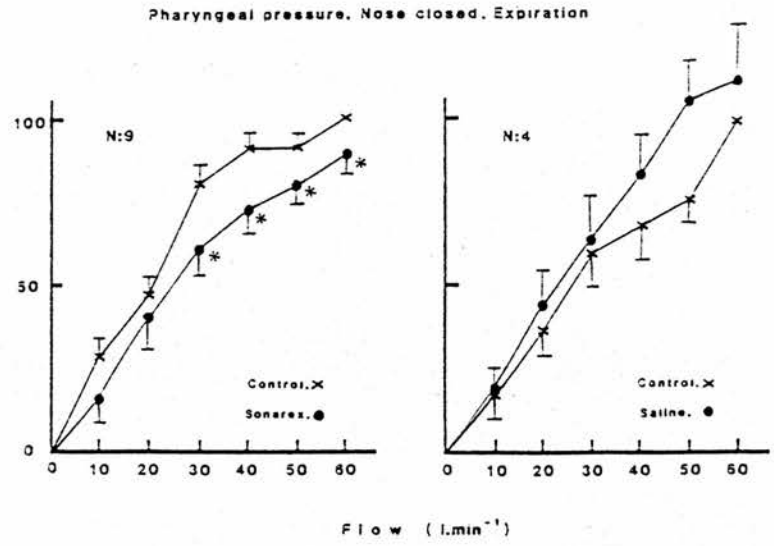

Fig. 4. – Averaged and normalized curves for flow/pressure relationships for pressures measured with the nose closed and in the expiratory phase. On the left are shown averaged curves for controls (crosses) and after Sonarex (filled circles); on the right are shown curves for controls (crosses) and after application of saline (filled circles). Vertical lines are SEMS. * $p<0.05$ for paired values for Sonarex and saline compared with controls.

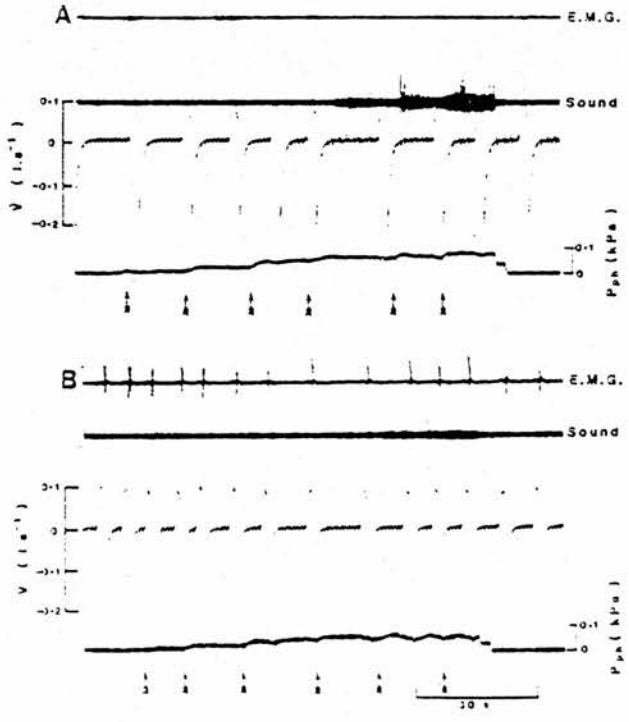

Fig. 5. – Effects of Sonarex on sound produced by airflow through the upper airways isolated *in situ* with the nose open. From above down: genioglossus EMG, sound from a microphone next to the mouth, airflow (V) and pharyngeal pressure (Ppn). Flow was increased by steps of 10 l-min^{-1} at each arrow, therefore increasing up to a maximum of 60 l-min^{-1} for the furthest right-hand arrows. In (A), when airflow surpassed 40 l-min^{-1}, sound started to be recorded reaching a maximum at 60 l-min^{-1}. In (B), after administration of 0.5 ml of Sonarex into the pharynx, the same increments in airflow caused far less sound, starting at about 50 l-min^{-1}, and the pressure increases due to flow were smaller.

relationship produced a conspicuous sound at flows greater than 40 l·min⁻¹. In (B), after administration of Sonarex there was a smaller increase in pharyngeal pressure for each increment of flow and the sound was far smaller.

The effects of Sonarex and saline on the integrated sound during flow/pressure determinations of upper airways resistances were averaged (table 2). Sonarex decreased sound in each of the nine tests by a mean of -65% when the nose was open, and by -47% (n=7) when the nose was closed. By contrast, saline increased sound by 10% when the nose was open and by 55% when the nose was closed.

Discussion

We had hoped to conduct a blind cross-over study, but this proved impractical. The experimenter could distinguish between Sonarex and saline, since the former was slightly opalescent and its bubbles were stable. An attempt at a cross-over study did not give equal N-values for some variables. Pharyngeal pressure was often difficult to measure in tests with saline, although Sonarex invariably led to stable pharyngeal pressure records, presumably because of its lubricant and surface activities in the catheter and oropharynx. In dogs and conditions when there was no sound of snoring, or no genioglossus EMG, analysis of these variables was impossible. We have therefore included all results in the tabular analysis.

Sonarex is a proprietary treatment for snoring, subjects being instructed to instill four drops into each nostril (total about 0.5 ml) before sleeping [6, 7]. We found that it reduced upper airways resistance, decreased the sound of snoring, and increased genioglossus muscle activity. Some of these effects could be caused by changes in mechanical properties of the airways. For example, Sonarex could lower surface tension of any mucus or liquid lining the pharynx and would presumably reduce the adhesiveness of the pharyngeal soft tissues. The opening and closing pressures of the pharynx of experimental animals and dead humans are influenced by tissue adhesiveness [8, 9]. In an important study in man, phosphocholinamin (a surfactant consisting of lecithin in mineral oil) reduced the degree and frequency of occurrence of snoring [10]; upper airways resistances were not measured. It is not known to what extent the intensity and quality of snoring depend on the amount of secretions in the upper airways and on their rheology but, if these properties were changed by surface-active materials, snoring might also be affected. It is interesting that saline had the opposite effect to Sonarex on snoring; integrated sound was increased and the subjective impression was that this change was because of the introduction of a "bubbling" noise not heard after Sonarex.

Genioglossus activity was increased by Sonarex, which strongly suggests that a reflex was activated leading to greater pharyngeal dilatation. Reflexes from the upper airways which contract the pharyngeal dilator muscles are well established [3–5, 11, 12], although little is known about the natural stimuli to the nervous receptors that mediate them. The ingredients of Sonarex might have had a direct action on nervous receptors, or the reflexes might be influenced secondarily by induced mechanical changes. Genioglossus activity increased in both inspiratory and expiratory phases, consistent with the measured changes in upper airways resistances. Other studies have shown that the genioglossus, although an airway dilator muscle, can discharge in one or both respiratory phases depending on the position of the tongue [13, 14].

We have not attempted to identify which of the ingredients of Sonarex are active. Polysorbate 80 is a non-ionic surfactant which, in high concentrations, changes the permeability of rabbit oral mucosa [15] and removes lipids from the surface of the intestine [16]. It also increases the permeability of the intestine to small solutes [17]. Benzalkonium chloride is a cationic surfactant that increases the permeability of the intestine to small solutes [17–19] and changes the ultrastructure of the cornea [20]. Thus, both agents might work not only by their mechanical surface activity but also by altering epithelial function. There is little value in comparing concentrations with different methods, since the concentrations in our studies could only be determined by direct experiment or by knowing the dilution factor of the Sonarex added to any secretions already in the upper airways. With regard to glycerol, we have found no evidence that it might have either appreciable surface activity or a physiological effect on epithelia.

One important observation was that 0.9% sodium chloride solution was active in some respects. It frequently lowered upper airways resistance and increased genioglossus EMG, although considerably less than Sonarex. It did not, like Sonarex, decrease the sound of snoring but increased it; this may indicate that the surface activity of Sonarex is the more important factor influencing the sound of snoring. Our difficulty in interpreting these results is due to ignorance of the chemical and physical properties of the resting secretions in the pharynx. There could be considerable dehydration, especially if breathing is through the mouth as occurs in many snoring subjects. Thus, not only could the mucus be "thicker and stickier" than normal, but the osmolarity of the epithelial fluid could be higher than that of 0.9% saline. 0.9% saline could have an action on the adhesiveness of mucus and of the airway soft tissue, and could have reflex actions in the nose and the larynx [4, 21]. An airway which had become acclimatized to epithelial fluid of high osmolarity due to evaporation might well respond to the introduction of "normal" saline as a non-physiological event.

The actions of Sonarex cannot be explained as being due solely to its saline base for two reasons. Firstly, as indicated above, the effects of saline on resistance were usually smaller and more variable than those of Sonarex; they were certainly far smaller on genioglossus EMG and were in the opposite direction on snoring. Secondly, when flow/pressure curves were determined at 5–20 min after application of saline or Sonarex and compared to controls, Sonarex produced a significant reduction in resistance whereas saline increased resistance. Sonarex also markedly decreased the irregularities of the flow/

pressure curves. These irregularities may well be due to sudden changes in the position of the epiglottus and the soft palate as flow is increased through the upper airways [1]. At this time Sonarex also decreased the noise of airflow through the upper airways. Saline may not have directly increased resistance assessed from flow/pressure curves because we did no controls without administration of either saline or Sonarex. The passage of time between determination of flow/pressure curves might have been enough to increase resistance by a drying out of the upper airways.

In conclusion, our results show that an animal model for studying snoring and upper airways obstruction can be used successfully to test physiological mechanisms. The results support human studies indicating that surface-active agents in the upper airways can reduce snoring [10], and animal studies indicating that upper airways resistance depends upon soft tissue adhesiveness [8, 9] and on the contraction of airway dilator muscles [3, 4].

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RÉSUMÉ: Nous avons mesuré les résistances des voies aériennes supérieures, à la fois au niveau de la trachée et du pharynx, par rapport à l'atmosphère, ainsi que l'électromyogramme du muscle génioglosse et les bruits de ronflements chez des lévriers anesthésiés. Nous avons déterminé également les courbes débit-pression pour les voies aériennes supérieures, en utilisant un débit continu de production extra-corporelle et nous les avons analysées en terme de résistance au niveau de la trachée et du pharynx. Nous avons etudié l'effet d'une solution saline à 0.9% et celui du Sonarex (un mélange commercial contenant du chlorure sodique, du glycérol, du polysorbate 80 et du chlorure de benzalkonium) sur les variables mesurées. Quand les chiens respirent au travers des voies aériennes supérieures, la solution saline isotonique ainsi que le Sonarex diminuent les résistances des voies aériennes supérieures mais le Sonarex le fait de façon plus régulière. L'activité du génioglosse est augmentée et les bruits de ronflements diminuent avec le Sonarex. Quand les courbes débit-pression sont déterminées, on observe de 5 à 20 mn après Sonarex une diminution de la résistance des voies aériennes supérieures et une courbe plus lisse alors que l'inhalation de solution saline entraîne une augmentation de la résistance. Les bruits produits par un débit continu au travers de voies aériennes supérieures sont diminués par le Sonarex mais augmentés par la solution saline isotonique. La résistance des voies aériennes supérieures est diminuée aussi bien par le Sonarex que par la solution saline mais le Sonarex diminue en outre les bruits de ronflements ainsi que la résistance et le son entraînés par un débit continu d'air au travers des voies aériennes supérieures.

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The effect of high frequency ventilation on pattern of breathing.

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THE EFFECT OF HIGH-FREQUENCY VENTILATION ON PATTERN OF BREATHING OF ANAESTHETIZED RABBITS

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SUMMARY

The lungs of anaesthetized rabbits were ventilated with a frequency of 30 Hz and a displacement of 5 ml. High-frequency ventilation (HFV) was superimposed on static inflation or deflation pressures of 2·5 and 5 cmH_2O and was maintained for 10 s. Changes in pattern of breathing in response to this procedure were recorded before and during block of pulmonary stretch receptors by SO_2. With lung stretch receptors intact apnoea or extended duration of expiration demonstrated the predominant role of pulmonary stretch receptors in the response to HFV. Block of stretch receptors exposed effects of other pulmonary afferents, presumably rapidly adapting receptors, during HFV, and demonstrated the influence of their sustained stimulation on pattern of breathing as augmented breaths and the reduction of duration of expiration. High-frequency ventilation may represent a useful experimental method of stimulating rapidly adapting receptors.

INTRODUCTION

High-frequency ventilation (HFV), in the context of the present study, can be described as mechanical ventilation of the lungs with tidal volumes that approach the subject's dead space volume, and with frequencies of up to 30 Hz. It was originally devised to avoid the arterial blood pressure fluctuations produced by more conventional methods of artificial ventilation in animal experiments (Jonzon, Oberg, Sedin & Sjostrand, 1970) and later adopted as a means of reducing trauma produced by the pressures generated by more conventional methods of ventilation in patients with acute lung disease or air leak through a bronchopleural fistula (e.g. Carlon, Ray, Klain & McCormack, 1980).

A striking characteristic of HFV is its ability to produce apnoea which is not the result of hypocapnia (Butler, Bohn, Bryan & Froese, 1980; Thompson, Marchak, Bryan & Froese, 1980). This apnoea has been attributed to the increased activity of slowly adapting pulmonary stretch receptors (PSRs) in response to HFV (Wozniak, Davenport & Kosch, 1983), and by Sant'Ambrogio & Davenport (1986) in response to high-frequency oscillations of the tracheal wall. Slowly adapting PSRs are not the only receptors stimulated by HFV. Wozniak *et al.* (1983) recorded an increase in the activity of pulmonary rapidly adapting receptors (RAR). We and others (Davies & Roumy, 1982; Pack & DeLaney, 1983) have previously demonstrated that RAR are particularly sensitive to rapid changes in lung volume and that their reflex effect seems to be a shortening of the duration of expiration (T_E), production of augmented breaths, and the precipitation of the onset of inspiration (Davies, Sant'Ambrogio & Sant'Ambrogio, 1981). These reflexes are of a generally inspiratory-provoking nature. This, and the fact that Kohl & Koller (1984) recorded tonic phrenic discharge in rabbits subjected to HFV, suggests that respiratory reflexes other than those from PSRs may be active during HFV.

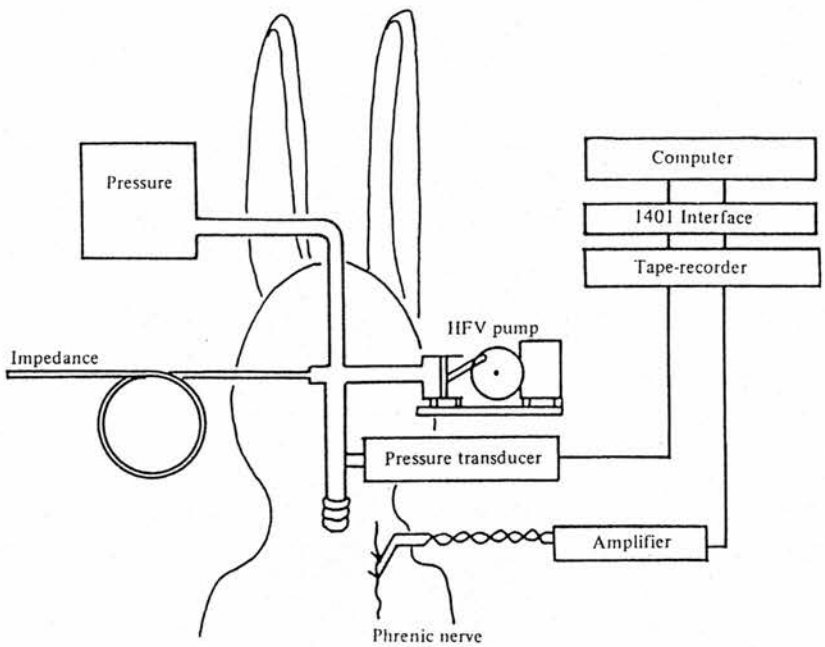

Fig. 1. Experimental set-up.

To investigate and define the nature of these reflexes we exposed anaesthetized rabbits to HFV before and during specific block of their PSRs, while inflating or deflating their lungs with a small static air pressure to increase or decrease stretch receptor activity.

METHODS

We used eight New Zealand White rabbits of either sex weighing between 2·0 and 3·5 kg. Anaesthesia was induced and maintained with sodium pentobarbitone, 40 mg kg^{-1} initial dose, and further doses given as necessary. A polyethylene cannula was tied into the trachea immediately below the larynx. Polyethylene catheters were inserted into a femoral artery and vein for measurement of arterial blood pressure and infusions respectively. Phrenic activity was recorded from multifibre strands of the central cut end of the upper root of the right phrenic nerve placed in a trough of liquid paraffin. Platinum electrodes and Neurolog amplifiers (N103 and N105) were used. The pattern of breathing in terms of duration of inspiration (T_I) and expiration (T_E) was measured from the initial increases and the starts of the rapid decreases of phrenic activity. Airway pressure and arterial blood pressure were measured by transducers (Honeywell 140PC) attached to the arterial cannula and to a side arm of the tracheal cannula (Fig. 1).

High-frequency ventilation (HFV) was produced by a reciprocating piston pump with a displacement of 5 ml and a frequency of 30 Hz attached to a manifold connected to the tracheal cannula. A polyethylene catheter 150 mm long and internal diameter 3 mm (dead space 10·6 ml; resistance 30 $cmH_2O\ l\ s^{-1}$) attached to another arm of the manifold provided an impedance to high-frequency movement of air which was therefore directed to the lungs. Low-frequency movement of air produced by spontaneous breathing was not impeded. Static pressure of $\pm 2{\cdot}5$ or 5 cmH_2O, was applied by a rotary pump connected to the manifold withdrawing or adding air through the impedance at the appropriate rate. The manifold was connected to the tracheal cannula and approximately ten control breaths were recorded before pressure and HFV were applied. HFV was superimposed on static inflation or deflation pressures of 5 cmH_2O for periods of 10 s only. HFV was

Table 1. *Changes in duration (mean ± S.E.M.) of inspiration (T_I) and expiration (T_E) produced by changes in airway pressure, above and below atmospheric, combined with high-frequency ventilation (HFV), before and during block of pulmonary stretch receptors (PSRs)*

			HFV			
	T_I	T_E	T_I	T_E	T_I	T_E
	With PSRs intact					
Airway pressure (cmH_2O)						
+5·0	+0·06 ± 0·07	+0·61 ± 0·50*	+0·08 ± 0·05	+2·45 ± 1·68*	+0·07 ± 0·05	+0·98 ± 0·55*
	$n = 19$		$n = 18$		$n = 24$	
+2·5	+0·03 ± 0·02	+0·24 ± 0·18*	+0·23 ± 0·18	+0·74 ± 0·68*	+0·08 ± 0·05	+0·41 ± 0·19*
	$n = 24$		$n = 16$		$n = 24$	
Atmospheric pressure			+0·24 ± 0·19	−0·24 ± 0·16*		
			$n = 22$			
−2·5	+0·03 ± 0·03	−0·34 ± 0·23*	+0·14 ± 0·11	−0·76 ± 0·22*	+0·09 ± 0·13	+0·11 ± 0·21*
	$n = 24$		$n = 24$		$n = 21$	
−5·0	−0·07 ± 0·06	−0·65 ± 0·35*	+0·08 ± 0·07	−1·33 ± 1·37*	+0·15 ± 0·10	−0·23 ± 0·26*
	$n = 24$		$n = 24$		$n = 24$	
	With PSRs blocked					
Airway pressure (cmH_2O)						
+5·0	−0·16 ± 0·12	−0·01 ± 0·07	+0·04 ± 0·17	−0·34 ± 0·36*	−0·02 ± 0·24	+0·29 ± 0·21
	$n = 24$		$n = 24$		$n = 24$	
+2·5	−0·08 ± 0·06	−0·03 ± 0·06	+0·20 ± 0·21	−0·49 ± 0·40*	+0·20 ± 0·18	+0·25 ± 0·19
	$n = 24$		$n = 21$		$n = 23$	
Atmospheric pressure			+0·21 ± 0·15	−0·31 ± 0·23*		
			$n = 23$			
−2·5	+0·10 ± 0·21	−0·03 ± 0·16	+0·48 ± 0·42	−0·32 ± 0·26*	+0·33 ± 0·16	+0·08 ± 0·19
	$n = 18$		$n = 18$		$n = 18$	
−5·0	+0·05 ± 0·07	−0·14 ± 0·15	+0·26 ± 0·26	−0·50 ± 0·50*	+0·40 ± 0·24	−0·03 ± 0·12
	$n = 20$		$n = 18$		$n = 18$	

* Indicates $P < 0·05$, that the effect was due to chance. Trials containing apnoea and augmented breaths are excluded.

applied before and during block of pulmonary stretch receptors, produced by causing the anaesthetized rabbits to breathe 200 p.p.m. sulphur dioxide gas for 10 min, (Davies, Dixon, Callanan Huszczuk, Widdicombe & Wise, 1978) and 15 min after bilateral cervical vagotomy.

Variables were recorded on magnetic tape (TEAC, XR30) and digitized by a Cambridge Electronics 1401 interface for storage on a computer hard disc (Tandon PCX20) for later analysis. Data were sampled in 20 ms bins and phrenic activity subjected to true digital integration by the computer before display. Wilcoxon's rank sum test was used to determine the statistical significance of changes in response to pressure and HFV. McNemar's test was applied to the difference of occurrence of augmented breaths and apnoeas before and after stretch receptor block. Differences were considered significant at a P value of 0·05. Results are given as mean ± S.E.M.

RESULTS

The effects of HFV on pattern of breathing depended on the degree and nature of static air pressure concomitantly applied to the lungs, and are illustrated in Fig. 2 and summarized in Table 1. The results in Table 1 are from trials in which apnoea did not occur. The occurrence of apnoeas and augmented breaths is given in each following section of the results. It will be seen from Fig. 2 that the pressure fluctuations as a result of HFV were of the order of 2 cmH_2O.

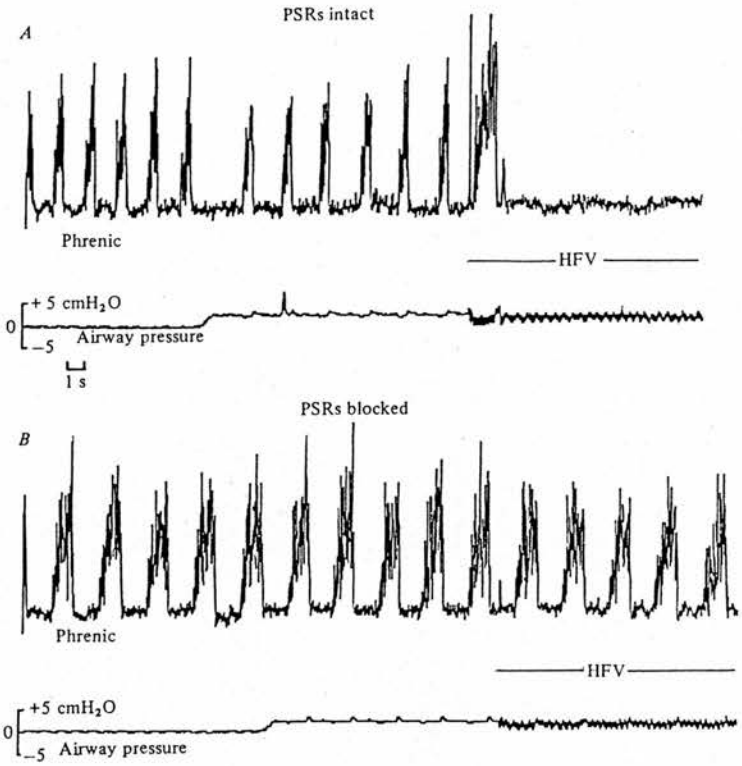

Fig. 2. The effect of 5 cmH_2O positive pressure and HFV applied to the airway of an anaesthetized rabbit on integrated phrenic activity and intra-airway pressure, before and during block of pulmonary stretch receptors (PSRs) by SO_2. Both signals were sampled in 20 ms bins. Note the augmented breath provoked by HFV when stretch receptors were intact.

Control breathing

The mean control T_I ($0{\cdot}71 \pm 0{\cdot}1$ s, $n = 120$) with stretch receptors intact was significantly different ($P < 0{\cdot}01$) from mean T_I with stretch receptors blocked($1{\cdot}44 \pm 0{\cdot}28$ s, $n = 120$). On the other hand the mean control and 'blocked' T_E($1{\cdot}44 \pm 0{\cdot}27$ s, $n = 120$ and $1{\cdot}56 \pm 0{\cdot}29$ s, $n = 120$) were not significantly different. Mean T_I and T_E for the first three breaths after HFV was stopped and static pressure removed, before ($T_I = 0{\cdot}76 \pm 0{\cdot}12$ s, $n = 116$; $T_E = 1{\cdot}47 \pm 0{\cdot}43$ s, $n = 116$) and during ($T_I = 1{\cdot}64 \pm 0{\cdot}38$ s, $n = 116$; $T_E = 1{\cdot}57 \pm 0{\cdot}39$ s, $n = 116$) PSR blockade were not significantly different from the control values before HFV and pressure were applied.

Effects at atmospheric pressure

With mean intra-tracheal pressure at or near atmospheric, HFV caused a statistically insignificant increase in T_I and significant decrease in T_E whether PSRs were intact or blocked (Table 1, Fig. 3). HFV at atmospheric pressure never produced apnoea, with stretch receptors intact or blocked, and produced two and one augmented breaths respectively with stretch receptors functional and blocked.

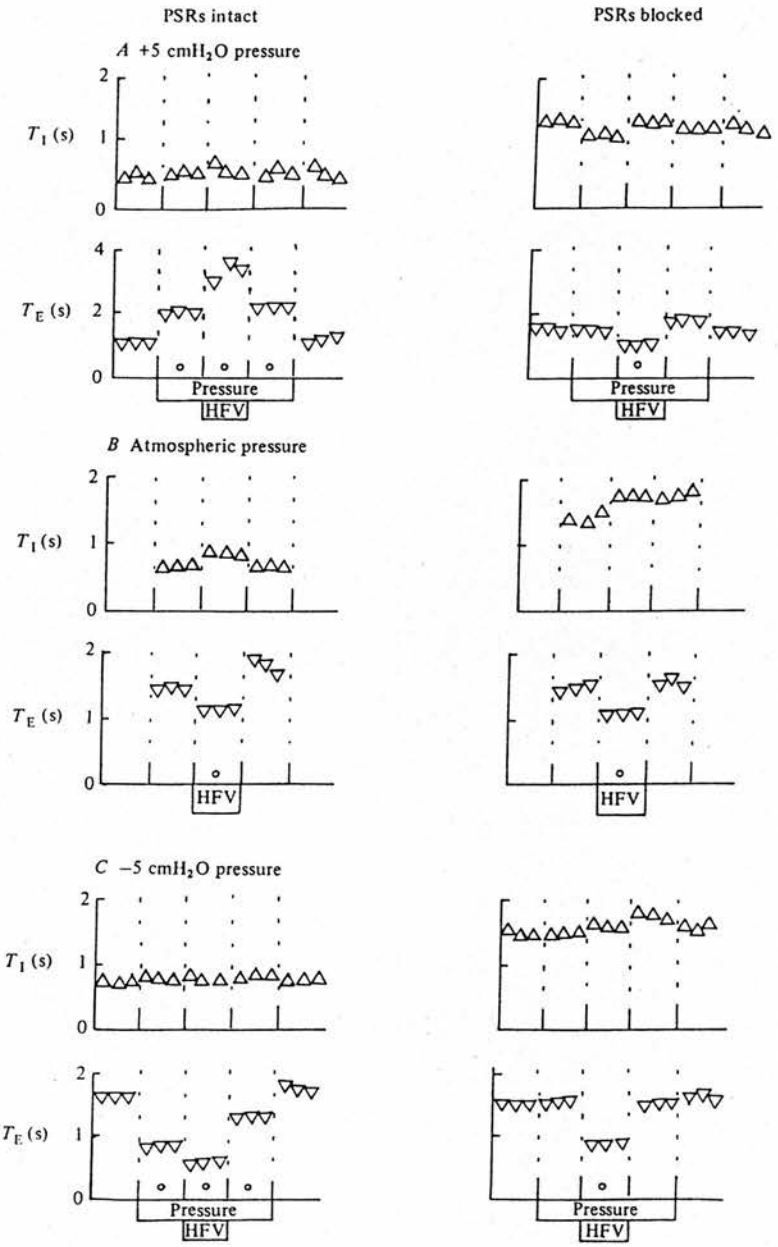

Fig. 3. Mean values of T_I (△) and T_E (▽) for the first three breaths from eight rabbits during exposure to atmospheric or increased or decreased intratracheal pressure and high-frequency ventilation (HFV). Runs containing apnoea and augmented breaths are excluded. Significant change from control value is indicated by ○.

Effects of positive pressure

The responses to positive pressure inflations of 2·5 and 5·0 cmH_2O differed only in magnitude and consisted of statistically insignificant increases in T_I, and significant increases in T_E before stretch receptor block. During blockade these changes in T_I and T_E were converted to reductions, neither of which reached statistical levels of significance (Table 1, Fig. 3). HFV applied during positive pressure inflation while stretch receptors were intact produced a significant increase in T_E with no significant change in T_I. Block of stretch receptors converted the increase in T_E to a decrease.

The combination of positive pressure and HFV produced apnoea in eight trials and augmented the first breath of six out of sixteen trials (two at 5·0 cmH_2O; four at 2·5 cmH_2O) with stretch receptors intact. With stretch receptors blocked apnoea never occurred and the first breath was augmented in one out of sixteen trials, showing a clear difference between the blocked and intact animals.

Effects of negative pressure

As with positive pressure the responses to deflation of the lungs by negative pressures of 2·5 and 5·0 cmH_2O differed only in magnitude and consisted of increases in T_I and statistically significant decreases in T_E. Unlike the effects of positive pressure the direction of the change in T_E was not altered by block of PSRs (Table 1, Fig. 3). HFV applied during negative pressure while stretch receptors were intact increased both the lengthening of T_I and the shortening of T_E at a statistically significant level. This effect survived block of PSRs.

The combination of negative pressure and HFV never produced apnoea and rarely provoked augmented breaths (one in sixteen trials). The single augmented breath occurred when stretch receptors were blocked.

Vagotomy

The effects of the positive and negative pressures and HFV could not be evoked 15 min after bilateral cervical vagotomy.

DISCUSSION

During HFV apnoea occurs in both animals and man. It has been concluded that this is probably due to stimulation of slowly adapting pulmonary stretch receptors (PSRs). It was not clear however whether other vagal pulmonary receptors (e.g. RARs) are stimulated sufficiently to provoke a reflex modulation of breathing in the presence of enhanced stretch receptor activity. In many species changes in the pattern of breathing in response to other types of mechanical stimulation led us to suspect this might be so. In particular Jonzon (1977) reported that each cycle of inflation during HFV augmented phrenic activity in cats and Kohl & Koller (1984) demonstrated persistent phrenic and diaphragmatic activity during HFV-induced apnoea, which may be due to the activation of rapidly adapting receptors.

With regard to type J receptors (Paintal, 1969) and C fibre endings (Coleridge & Coleridge, 1977) in cats, inflations of up to several times tidal volume failed to excite J receptors and in rabbits they are either not stimulated, or only very weakly, by large maintained inflations or deflations (Sellick & Widdicombe, 1970). Recently Pisarri, Jonzon, Schultz, Coleridge & Coleridge (1987) have demonstrated by direct recording that

pulmonary C fibres are not stimulated by HFV. Supporting evidence for the absence of C fibre stimulation by HFV comes from the effect of vagal cooling on tracheal gland secretion during HFV (Schultz, Jonzon, Pisarri, Goodman, Davis, Coleridge & Coleridge, 1987).

The wave form of the HFV applied to the lungs is clearly of some consequence. In the present study the pressure generated approximated to a sine wave. Other workers (e.g. Homma, Onimaru, Oouchi & Ichikawa, 1985) have used rapidly repeated ramps of inflation or deflation. This form of HFV may be considered as a high-frequency series of pulses of inflation or deflation; and their results are entirely in agreement, both in terms of reflexes (Homma, Isobe, Onimaru & Oouchi, 1986) and receptor activity (Homma, Isobe, Iwase, Onimaru & Sibuya, 1987) with those we obtained with single pulses in previous experiments (Davies & Roumy, 1982).

The apnoea produced by HFV has been attributed to causes other than pulmonary receptor activity. A lower $P_{a.CO_2}$ during HFV certainly promotes apnoea (Zwart, Jansen & Versprille, 1981) and it has been suggested that extravagal afferents are involved (Butler *et al.* 1980; Thompson *et al.* 1980). However, the speed of onset of apnoea and its abolition by vagotomy in the present and previous experiments (Kohl & Koller, 1984; Banzett, Reid & Lehir, 1985) demonstrate these factors are not essential for its production. To control the effect of changed blood gases in our experiments HFV trials were limited to a 10 s period.

Both by its similarity to the classical Hering–Breuer inflation reflex, and from direct recording in dogs (Sant'Ambrogio & Davenport, 1986) and rabbits (Kohl & Koller, 1984) it seems fairly clear that the apnoea of HFV is due to stimulation of slowly adapting PSRs. This is supported by our observations that block of PSRs abolishes the apnoea produced by static lung inflation or HFV. Unlike Pack, Davies, Marino & Fishman (1980) we could not demonstrate alterations in T_I as a result of HFV; an observation which agrees with that of Kohl & Koller (1984) and supports our previous finding of an intransigent T_I compared with T_E in response to a variety of stimuli (Davies & Kohl, 1982; Davies & Roumy, 1986). The apnoea of HFV is likely to be an exaggerated form of the extended T_E seen in those rabbits where apnoea did not occur. Apnoea may override reflex effects of other simultaneously stimulated pulmonary receptors. Man, Man & Kappagoda (1983) suggested, on the basis of experiments involving cold block of the vagi, that 'rapidly adapting receptors are not likely to have been activated'. However cold block of the whole cervical vagus is not as specific as the block produced by breathing SO_2. Thus the influence of rapidly adapting receptor activity may have been diminished in their experiments. Support for the suggestion that RAR activity could influence pattern of breathing in our present experiments comes from the effect produced by blocking PSRs on T_E and the occurrence of augmented breaths. Both shortening of T_E and production of augmented breaths have been attributed to RAR activity (Davies & Roumy, 1982).

The interaction between static positive or negative pressure and HFV (Fig. 2, Table 1) may demonstrate the relative contributions of PSR and other (probably mainly RAR) activity to control of pattern of breathing when functional residual capacity is shifted. The differences in effect of HFV under conditions of lung inflation or deflation may also give some insight into its mechanism of stimulation of receptors. A similar suggestion has been made by Sant'Ambrogio & Davenport (1986) in relation to bronchomotor tone. Stiffening the lungs with a slight inflation pressure may thus increase the stimulation of PSRs to a level which overpowers the influence of RAR activity. It is unlikely that with the positive pressures involved the amplitude of movement of the lungs resulting from HFV was much reduced from that at atmospheric pressure; thus the augmenting effect of inflation

on HFV may be the result of more effective transmission of the mechanical stimulus to the PSRs. Wozniak *et al.* (1983) have recorded RAR activity and found it to be stimulated by HFV. The reflex expression of this stimulation would not be seen in our experiments until the overpowering effects of PSRs were removed by SO_2 block or RAR activity reached a level that 'broke through' the PSR effects in the form of an augmented breath. Davies & Roumy (1982) have previously remarked on the inability of RAR activity to influence T_I directly except in the form of provoking a single augmented breath. This phenomenon was seen again in the present series of experiments. The incidence of augmented breaths during HFV increased with inflating pressure of the lungs. This may reflect the permissive role stretch receptors play in respiratory reflexes such as cough (Sant'Ambrogio, Sant'Ambrogio & Davies, 1984) and augmented breaths provoked by other means (Davies & Roumy, 1982).

In summary, our results support the suggestion that the apnoea produced by HFV is the result of stimulation of PSRs alone. We cannot, however, say that PSRs are the only pulmonary vagal afferents stimulated by HFV. The reflex effects of such stimulation were exposed by block of PSRs. It seems that high-frequency ventilation provides a convenient and rapidly reversible method of stimulating pulmonary rapidly adapting receptors. It offers the unique facility of a stimulus of this type of receptor that can be sustained undiminished for a considerable length of time. Used in conjunction with a highly specific block of pulmonary stretch receptors it should provide a useful tool for the investigation of the reflex cardiovascular (Marshall & Metcalfe, 1986) and respiratory effects of rapidly adapting pulmonary receptors.

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Publication 47.

Davies,A. & Pack,R.J.(1990)

Lung reflexes in anaesthetized rabbits with pulmonary fibrosis.

J.Physiol.420,67P.

Lung reflexes in anaesthetized rabbits with pulmonary fibrosis

BY A. DAVIES and R. J. PACK*. *Departments of Physiology, University Medical School, Teviot Place, Edinburgh EH8 9AG and *Massey University, Palmerston North, New Zealand*

Pulmonary fibrosis was induced in five New Zealand White Rabbits (Derks & Jacobovitz-Derks, 1977) by repeated injections of oleic acid (0·1 ml kg^{-1}). They were then anesthetized with I.V. sodium pentabarbitone (40 mg kg^{-1} induction dose). Breathing, intrapleural pressure, lung mechanics and afferent activity from slowly and rapidly adapting receptors in their lungs were recorded. Lung reflexes were produced by sustained inflations and deflations and by pulses (100 ms duration) of positive or negative pressure. The response to inhaled carbon dioxide was measured. Pulmonary stretch receptor activity was then inhibited (Davies *et al.* 1978). The lung reflexes and response to CO_2 were elicited again and finally 15 min after both vagi had been cut.

The minute ventilation response to elevation of end tidal CO_2 in the fibrotic rabbits was increased in the intact but not in the stretch receptor blocked or vagotomised case. The reflex changes in the duration of expiration were more profoundly modified than the changes in duration of inspiration in the fibrotic rabbits. These differences were not abolished by the inhibition of stretch receptor activity.

Histological examination of the lungs revealed that they fulfilled Carrington's (1968) criteria for diffuse interstitial pulmonary fibrosis. The changes in sensitivity to inhaled CO_2 can be interpreted as being produced by changes in rest minute ventilation in the fibrotic rabbits while the changed sensitivity to brief or sustained changes in lung volume can be interpreted as being produced by changes in pulmonary receptor activity itself, probably produced by changes in the mechanical properties of lung tissue surrounding the receptors. This receptor activity is being analysed.

Supported by the MRC.

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Publication 48.

Davies,A.(1989)

Review of "Respiratory Function of the Upper Airway"
By Mathews & Sant'Ambrogio.

For Q.J.Exper.Physiol.74(2),229-230.

Respiratory Function of the Upper Airway (volume 35 in *Lung Biology in Health and Disease* series). Edited by OOMMEN P. MATHEW and GIUSEPPE SANT'AMBROGIO. Pp. 645. (Marcel Dekker, 1988.) $125.00, $150.00 outside USA and Canada. ISBN 0 8247 7802 2.

As this is the thirty-fifth volume in the series *Lung Biology in Health and Disease* one might expect its contributors to have some difficulty in finding new perspectives from which to view the subject. Not so. Although there are inevitably aspects in this volume which have been touched upon in previous parts of the series, the contributors give us some new insights into that region of the respiratory system which is now attracting much attention, due to the upsurge of interest in sleep apnoea and sudden infant death syndrome over the last decade.

The volume opens with an historical perspective and the biblical lesson beloved of respiratory physiologists (Genesis 2:7). We are also told that the harmful effects of snoring were recognized as early as the 19th century, which perhaps implies the publication of this book is very much overdue. Comparative aspects of the upper airway are dealt with in a chapter whose only fault is its brevity. The author moves from waterways to airways in eight fascinating pages, a number which I would have been happy to see expanded. Functional anatomy of the upper airway during development is beautifully dealt with, largely by high-quality informative line drawings. It may be a reflection of the greater certainty with which anatomists can view the world that the section on 'Speculation' on this subject is left to the last two pages. Next the reader is alerted to the distinct nature of the upper airway muscles and then their central control. This latter section is a paradigm of what contributions to a book of this type should be. The author vigorously stimulates his reader with most pertinent questions. If, as might be supposed, the amount of space devoted to an aspect of a subject within a chapter reflects the received wisdom on that aspect then the chapter on nerve receptors of the upper airway indicates how little is known about the nose and pharynx compared to the larynx. The brevity of the section on the extrathoracic trachea suggests, however, that the authors, with many years of leading work in this area, and the fiat of editorial control at their disposal have not indulged themselves, or us, with a more expanded version. The next section deals with nasal and pharyngeal reflexes and with the problems of identifying their components, exacerbated by non-specificity of receptors, and the fact that most stimuli will act on several sites and several afferent pathways. The need for more pharmacological studies of this region is pointed out. The chapter on laryngeal reflexes is to a certain extent inevitably linked to the earlier chapter on nerve receptors of the upper airway by the similarity of stimuli which are dealt with, the authors completing the arc in this section. The great difference between adult and neonate is brought out in a chapter on upper airway reflexes in newborns. We are regaled by interesting information of a specific and general nature. Apparently it is uncommon to see newborn babies sneeze or cough despite evidence that they could cough 'if they wanted to'. Which leads to the question of why infants generally respond with apnoea to stimuli which provoke cough in the adult. This fascinating chapter informs the reader what parents know only too well: 'in the human infant expulsive regurgitation is very common' and confirms our suspicion that it is an 'active mechanism'. The chapter on a biomechanical view of upper airway function suggests to me that the hard questions in fluid dynamics posed by the intrathoracic airway are relatively simple when compared with the questions posed by the upper airway. The origins of nasal airflow resistance are then described and this section is followed by a description of the modification of inspired air. We are told that inspired air reaches an identical condition in the subglotic region during either nose or mouth breathing at rest; which may help to allay some of the anxieties earlier chapters may have aroused about sleeping with one's mouth open. The role of the upper airway in control of respiratory flow and lung volume in humans is next described, almost exclusively in adults. Behaviour of the upper airway in a number of respiratory diseases concludes this section. The link between respiration and sound production is dealt with in a highly informative chapter whose easy peripatetic style makes it most pleasant reading. A section on regulation of breathing pattern during feeding directs our attention largely towards the newborn. The author dilates on the correct method of characterizing breathing pattern to best deal with the interruptions in air flow that are a major feature of this phenomenon and concludes with a section on disorders of sucking and swallowing. The upper airway is the first part of the respiratory tract to suffer the acute effects of cigarette smoke and the final chapter in this volume, on that subject, is entirely appropriate.

The series *Lung Biology in Health and Disease* comprises thirty-five volumes, with six more in preparation. The present volume makes a significant contribution to this encyclopaedic treatment of the subject. There is no reason why monographs of this type should be as turgid and difficult as they sometimes are. This volume avoids this by a pleasant variety of pace, style and direction through its sixteen chapters. Personal preferences lead me to wish that some of the contributors could have been encouraged to expand their contributions, perhaps at the expense of the number of topics covered, but that is a council of perfection to editors who have struck a very acceptable balance.

I feel it is part of a reviewer's duty to recommend, or otherwise, the purchase of the book he reviews. At the price the volumes in this series command this would be a purely theoretical recommendation for individual, at least British individual, academics. However, any School of Biology or Medicine or Department thereof with pretensions towards the subject will find this an essential guide to the respiratory function of the upper airway.

A. DAVIES

Publication 49.

Davies,A.& Pack,R.J.(1990)

Lung receptor activity in anaesthetized rabbits with pulmonary fibrosis.

J.Physiol.422,25P..

Lung receptor activity in anaesthetized rabbits with pulmonary fibrosis

BY A. DAVIES and R. J. PACK*. *Departments of Physiology, University Medical School, Teviot Place, Edinburgh EH8 9AG and *Massey University, Palmerston North, New Zealand*

In five New Zealand White rabbits pulmonary fibrosis was induced by the method of Derks & Jacobovitz-Derks (1977). They were then anaesthetized with I.V. sodium pentobarbitone (40 mg/kg induction dose). Breathing, intrapleural pressure, lung mechanics and afferent activity from slowly adapting (PSR) and rapidly adapting (RAR) receptors in their lungs were recorded. Lung reflexes were produced by sustained inflations and deflations and by pulses (100 ms duration) of positive or negative pressure while recording from individual receptors in the lungs.

The number and behaviour of the two types of receptors were found to be different in the two groups of animals. For example, in the control animals 3 out of 31 receptors recorded showed discharges of the RAR type, while in the fibrotic animals 11 out of 41 receptors showed this pattern. The average PSR discharge (impulses $s^{-1} \pm$ S.D. 5 fibres in 5 rabbits) was 49 ± 29 in controls and 93 ± 45 in fibrotics; peak discharges were 72 ± 48 and 126 ± 72 respectively. Details of the differences in discharge pattern between the control and fibrotic animals will be described in this communication. These differences are probably due to changes in the mechanical properties of the lung tissue surrounding the receptors (compliance in controls $7{\cdot}2 \pm 0{\cdot}5$ and fibrosed $6{\cdot}1 \pm 0{\cdot}4$ ml cm H_2O^{-1}, mean $\pm$ S.E.M.), which in turn result from the development of lesions which conform histologically to Carrington's (1968) criteria for diffuse interstitial pulmonary fibrosis.

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Publication 50.

Davies,A. Elton,R.,Powers,N., Sara,T., Walsh,E.G.& Wright,G.W.(1990)

Human physiological tremor-effects of smoking cigarettes or drinking coffee.

J.Physiol.423,70P.

Human physiological tremor – effect of smoking cigarettes or drinking coffee

By A. Davies, R. Elton, N. Powers, T. Saba, E. G. Walsh and G. W. Wright.
Department of Physiology and Medical Statistics Unit, Edinburgh University, EH8 9AG

Some authors have stated that smoking or drinking coffee increase finger tremor but these claims have also been disputed. We have experimentally reinvestigated the problem using an accelerometer attached to a finger held horizontally. The method was thus isotonic. The signal was filtered to exclude frequencies outwith the band 7–12 Hz and integrated over 5 s. In a survey we undertook of 180 medical students the small number of smokers had no more tremor than the non-smokers and there was no correlation between estimated caffeine intake and tremor. Heavy smokers were asked to smoke two cigarettes after at least 2 h of abstinence and the measurement was then repeated. In the coffee experiments two cups of Nescafé were taken (270 mg caffeine) and the measurements made 45 min later. The results are given in Table 1.

Table 1 Mean and S.D. of tremor levels after smoking and drinking coffee

		Accelerometer ($cm\ s^{-1}\ s^{-1}$)			
	n	Control	Afterwards	*P*	Confidence limits
Cigarettes	25	$31{\cdot}6 \pm 16{\cdot}4$	$37{\cdot}1 \pm 17{\cdot}7$	$< 0{\cdot}05$	+1%, +37%
Coffee	14	$30{\cdot}9 \pm 12{\cdot}7$	$34{\cdot}5 \pm 19{\cdot}5$	n.s.	−13, +32%

(Wilcoxon signed ranks test)

T.S. is a medical student supported by the Wellcome Trust.

Publication 51.

Davies,A. (1991)

Impact and irritation in tobacco smoking.

Consultancy report for British-American Tobacco Co.

An Investigation of the Effects of the Two Isomers of Nicotine on Pulmonary Rapidly Adapting Receptors in Relation to the Sensation of Impact.

By

A.Davies

University Medical School Edinburgh

For

British and American Tobacco
Fundamental Research Unit
Southamton.

Introduction

It has been reported that only one of the two isomers of nicotine produces the subjective sensation of "impact".
This difference between the two types of nicotine offers the opportunity to test the hypothesis that nicotine stimulation of rapidly adapting (irritant) receptors is the origin of the sensation of impact.
Preliminary experiments in this laboratory, and by Dr.M.Dixon at B.A.T. have indicated that impact arises from sites high in the tracheobronchial tree. The time-course of the onset of impact suggests receptors from which it originates must be superficial (probably epithelial) in the airways. Rapidly adapting epithelial receptors exist from the nose to intrapulmonary airways. They are not however homogenious in their specific stimuli or afferent pathways.
Those in the lungs send their sensory information to the brain in the vagus nerves. Those in the larynx and very uppermost trachea have their afferent axons in the superior laryngeal nerve.
Because they are more accessible to investigation it was decided to first determine the responses of intrapulmonary rapidly adapting receptors to the two isomers of nicotine. As a result of the technique used a large number of slowly adapting receptors were available for recording, results for this type of receptor are therefore also briefly presented.

Methods

Smoke used in these experiments was obtained from three types of cigarette C, S and R. The composition of these cigarettes was unknown.

Five rats of either sex, weighing between 250 and 300gm were anaesthetized with an intraperitoneal injection of urethane (6ml 25% per kg.) A tracheal cannula was inserted 5mm below the larynx and extended to within approximately 1cm of the carina. The free end of the cannula was Y shaped.

An air filled stethograph was placed round the rats thorax to record breathing movements.

The left vagus nerve was dissected clear of tissue and cut high in the neck. The peripheral cut end was placed in a tray of liquid paraffin and single neural units dissected out. The activity of these units was picked up by silver electrodes and amplified. The amplified signal together with that from the stethograph was recorded on magnetic tape for later analysis by computer.
The type of receptor associated with each single unit identified was catagorised as slowly or rapidly adapting by its response to an inflation and then deflation pressure of 1 kPa. applied to the air in the lungs by connecting the tracheal cannula to a large drum maintained at the required pressure.

Activity from a receptor was recorded for 30s. 10ml. of smoke was then drawn from cigarette C, S or R into a glass syringe and diluted with a further 10ml. of room air. The dilute smoke was injected into one of the arms of the tracheal cannula over a period of 15s.
The procedure was repeated for the two other types of cigarette. The three types of smoke were administered three time each in random order, i.e. nine times in all. To avoid tachyphylaxis only two rapidly adapting receptors and one slowly adapting receptor were dosed in each rat.

Receptor activity was recorded for 60s. from the onset of administration of smoke. Activity was analysed in terms of the variables shown in Fig.2. for 30s. of control breathing, the first 30s. after application of smoke and a subsequent 30s.

Results

In each rat, for each fibre, 30s intervals before, immediately after and 30s after the application of tobaco smoke were analysed.
The interspike interval and stethograph output were digitised and displayed on a computer screen (Akhter386-25). Fig.1.is a "screen-dump" of part of an experimental run. Sections of the whole run were then selected for analysis by a custom written program which analysed activity in terms of the variables shown in Fig2.

Rapidly Adapting Receptors

Treating each of the 10 fibres as its own control it was found that there was a significant ($p<0.05$) increase in both peak frequency of discharge and the total number of action potentials in both the first and second 30s periods after application of smoke. There was no significant difference in the response to different types of smoke. Table 1. is a printout of the total numbers of action potentials recorded from the rapidly adapting receptors over these periods.

Slowly Adapting Receptors

Treating each of the 5 fibres as its own control it was found that there was an increase in peak frequency of discharge and total number of action potentials in both the first and second 30s periods after application of smoke. This was only significant ($p<0.05$) in the second 30s. There was no significant difference in the response to different types of smoke.

Discussion

Because of the rapid time-course of events in the sensation of impact it is unlikely that pulmonary stretch receptors are involved. However a small sample of slowly adapting receptors were recorded from in these experiments and the results will be briefly discussed.

The significant increase in peak frequency and total number of action potentials per unit time produced by both nicotine smokes and the nicotine free smoke (which ever that might have been) suggests that a major part of the stimulation of slowly adapting stretch receptors was the result of bronchoconstriction, which is a well documented effect of inhaled smoke (Nadel & Comro, 1961). Pulmonary stretch receptors lie in series with the smooth muscle of the bronchial tree and increased bronchomotor-tone would explain part or all of the increase in frequency seen. It is not

to be expected that the concentrations of smoke used in these experiments would have its major effect directly on the receptors because of their anatomical situation. However Hartiala, Mapp, Mitchell & Gould(1985) have clearly shown that blood born products of tobacco smoke (mainly nicotine) increase breathing and smooth-muscle tone. This is a direct (Takayanagi,Kizawa & Sone,1984) as well as reflex phenomena(Nadel & Widdicombe,1962).

The effects of inflating and deflating pressures on airways containing slowly adapting receptors have different effects depending on whether the airways are intra- or extrathoracically situated. This depends on the anatomical arrangement of the smooth muscle. Thus receptors may be stimulated by inflation or deflation pressures in the trachea (Sant.Ambrogio,1982). Similarly rapidly adapting receptors in the trachea and upper airways cause cough when stimulated, while those in the lungs produce rapid shallow breathing. The sensations produced are also different, cough being produced by stimulation of the upper airways while touching the epithelium of the lower airways produces a burning sensation which might be akin to "impact.

The action of certain irritants is very species specific (Rev:Sant'Ambrogio,1982)with ammonia and cigarette smoke being much more effective in stimulating receptors in rabbits than those in dogs for example. Our results support the findings of many other workers in the nature of the response to smoke and do not demonstrate a difference in the activity produced by either of the two smokes containing different isomers of nicotine.

Our experiments were designed to exclude the larynx from contact with the smoke. Lee,SantAmbrogio, Mathew & SantAmbrogio(1987) have demonstrated a complex response of laryngeal receptors to contact with tobacco smoke, including modulation of cold receptors. Cold is a sensation smokers say they experience on inhaling smoke.

Accepting the apocryphal reports that only one isomer of nicotine produces the sensation of impact, and the absence of difference we record between the two isomers

Leaves the larynx as the most likely site for the origin of impact.

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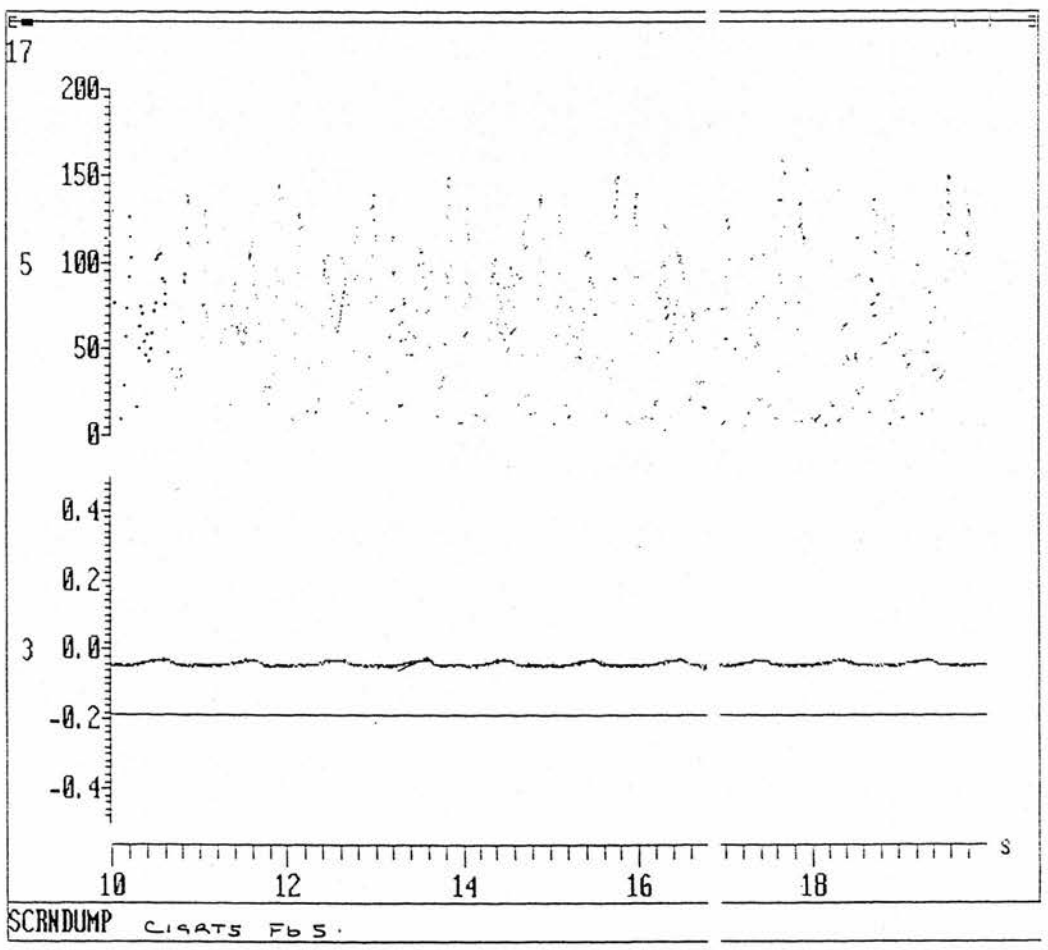
FIG. 1.

Screen-dump of part of an experimental run showing
Channel 5-Interspike intervals.
Channel 3- Stethograph output.



Publication 52.

Moores,C. & Davies,A.(1991)

Effect of high-frequency oscillation-.

J.Physiol.438p,59,1991

The effect of high frequency oscillation on the non-Newtonian properties of sputum

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Interest has recently been focussed on the possibility of increasing respiratory mucus clearance in disease by vibrating the air in the airways at a frequency of about 10–20 Hz and low tidal volume (high frequency ventilation, HFV). Some workers have demonstrated that clearance may be improved (George *et al.* 1984) while others have not (McEvoy *et al.* 1982). Effects on clearance could depend on changes in rheology of the mucus which has thixotropic properties.

Some investigations of this theory have used methods of measuring viscosity suitable for Newtonian liquids on highly non-Newtonian mucus. We subjected expectorated human bronchitic sputum, which owes its rheological properties largely to bronchial mucus, to *in vitro* high frequency oscillation to simulate HFV for various periods of time. The effect on its rheology was measured using a parallel plate rheometer and procedures suitable for non-Newtonian liquids. These included measurement of viscosity between two parallel plates oscillating through a very small arc at different frequencies, and measurement of strain under a very low stress.

Table 1. Effects of HFV on sputum viscosity

Viscosity during oscillation(as mean of 8 samples ± S.E.M.)

Frequency of oscillation (Hz)	Viscosity η (P)	Change in η after HFV for	
	No HFV	2 min	4 min
1	2.02 ± 0.97	0.77 ± 0.55	1.21 ± 0.47
8	0.05 ± 0.02	0.12 ± 0.06	0.20 ± 0.12

Strain under constant stress

No HFV	2 min HFV	4 min HFV
34.12 ± 0.92	17.39 ± 3.82	15.15 ± 3.85

Our results suggest that HFV causes a slight increase in viscosity of sputum. Any improvement in clearance resulting from HFV is therefore probably brought about by a mechanism other than reduction in mucus viscosity.

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Lung reflexes and receptor activity in a rabbit model of pulmonary fibrosis.

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Lung

Lung Reflexes and Receptor Activity in a Rabbit Model of Pulmonary Fibrosis

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Abstract. Our objective was to see if activity of individual slowly and rapidly adapting pulmonary receptors was changed by pulmonary fibrosis.

Diffuse interstitial lung fibrosis of several weeks' standing was induced in 8 rabbits. They displayed changes in lung mechanics and patterns of breathing, when compared to control rabbits, similar to those seen in patients who develop pulmonary fibrosis. Lung reflexes in the fibrotic rabbits were more profoundly changed than eupneic breathing in a way that could be interpreted as slowly adapting receptor activity, which was increased, being overpowered by a prepotent input from pulmonary rapidly adapting receptors. An increase in number of active rapidly adapting receptors was found in the fibrotic rabbits during direct vagal recording. We have demonstrated that pulmonary receptor activity is changed by lung fibrosis. It may be that these changes in receptor activity produce conflicting respiratory drives that could result in the sensation of dyspnea.

Key words: Breathing, control—Lung fibrosis—Lung reflexes—Lung receptors—Dyspnea.

Introduction

The role of pulmonary receptors in the control of pattern of breathing has been extensively investigated in conscious and anesthetized animals and humans [reviewed in 5]. It is also generally accepted that the activity of the 3 types of pulmonary receptor is probably changed in patients with respiratory disease [15, 19] as a result of changes in their physical and chemical environment [2, 3]. These changes may be the origin of the disordered

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patterns of breathing and dyspnea seen in patients with pulmonary fibrosis [16, 24].

Many animal models of human respiratory disease have been produced. Records of multifiber vagal afferent activity and vagal blocks of various specificities have been used in these models, and in cases of more acute damage to the lungs, to investigate changes in reflex neural control of breathing in lung disease. However, the activity of *individual* pulmonary receptors and the relative incidence of the types of receptor in disease models have not previously been quantified.

In the present study we produced diffuse interstitial lung fibrosis in rabbits and compared the incidence and activity of slowly and rapidly adapting pulmonary receptors with that in healthy rabbits.

Methods

Production of Fibrosis

Lung fibrosis was produced in 8 New Zealand White rabbits by an intravenous injection of 0.1 ml/kg of oleic acid (Aldrich Chemicals, London, UK) given at weekly intervals for 5 weeks to each rabbit. The final injection was followed by a period of 5 weeks to allow the fibrosis to develop. The mean weight of the rabbits was 2.61 ± 0.06 kg at the beginning of the procedure and 3.47 ± 0.08 kg at the end. No rabbit failed to gain weight or showed any signs of distress throughout the procedure.

Acute Experimental Procedure

The 8 fibrotic rabbits described above and 8 control rabbits weighing between 2.5 and 3.5 kg were anesthetized with an intravenous injection of 30 mg/kg sodium pentobarbitone.

Catheters were tied into the left femoral artery and vein. Supplementary doses of pentobarbitone were given via the venous catheter to maintain surgical anesthesia. Airflow was recorded by a Fleisch pneumotachograph head connected to the tracheal cannula. Tidal volume was obtained by integrating flow electronically. Transpulmonary pressure was measured by a differential transducer connected between a catheter inserted into a lower right intercostal space and the trachea. The airflow, tidal volume, and transpulmonary pressure signals were used to determine total lung resistance and compliance by the subtractor method [18], as modified by Nadel and Widdicombe [20]. Carbon dioxide in the respired air was monitored by an infrared gas analyzer (Beckman L.B.1), which sampled from the rostral side of the pneumotachograph head. After a period of control breathing, arterial partial pressure of carbon dioxide was increased by causing the rabbits to breathe approximately 4 and 6% carbon dioxide for 2 min. The carbon dioxide mixtures were prepared by mixing commercial carbon dioxide with air and passing the resulting mixture across the free end of the pneumotachograph head. The rabbit's responses to steps of maintained lung inflation or deflation (1 kPa [10 cmH_2O]) for several seconds, and pulses of inflation and deflation (2 kPa [20 cmH_2O]) for 100 ms were recorded. The pulses were produced by the method of Davies and Roumy [7] and manually synchronized with the beginning of inspiration.

Both vagus nerves were exposed in the midcervical region and loops of silk thread placed round them. Afferent vagal activity was recorded from the left vagus, which was cut high in the neck and the distal cut end placed in a copper tray filled with liquid paraffin. "Single fiber" preparations were made from strands of nerve that displayed respiratory rhythm when placed on a pair of silver wire electrodes. The receptors were classified as slowly or rapidly adapting according to their response to a step inflation of the lung by a pressure of 1 kPa (10 cmH_2O).

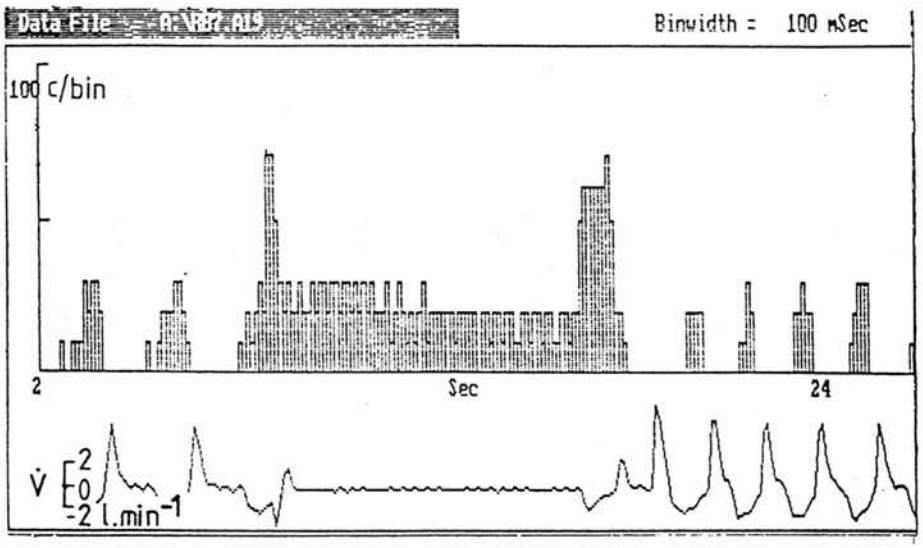

Fig. 1. Computer printout of pattern of discharge from a slowly adapting pulmonary "stretch" receptor during sustained inflation of the lungs. Number of action potentials in each 100 ms bin is plotted on the vertical axis of the upper trace. Respiratory air flow is plotted on the lower tracing.

Conduction velocity was initially used to differentiate between the slowly and rapidly adapting types of receptor, but it soon became apparent that the difference in properties was so marked that the criterion of a discharge that maintained more than 50% of its initial value after 2 s of lung inflation by 1 kPa (10 cmH_2O) was sufficiently sensitive to differentiate between the 2 types.

Electrical activity of the single fiber preparations was amplified by a high-gain RC amplifier (Neurolog) before being recorded, together with the other physiological variables, on magnetic tape by a TEAC XR-30 recorder. Patterns of discharge in response to sustained and pulsed inflation and deflation and increases in inhaled CO_2 were analyzed by dividing each breath into 100 ms bins and counting, by computer, the number of spikes in each bin (Fig. 1). These numbers, with their associated bin number, were stored for later analysis allowing a comparison between the normal and fibrotic rabbits.

The response to carbon dioxide was again recorded 10 min after bilateral vagotomy.

Statistical significance of difference between control and fibrotic animals, calculated by Student's unpaired t-test was taken as $p < 0.05$.

At the end of the experiment the rabbit was killed by an overdose of anesthetic. The trachea was clamped immediately below the larynx and the lungs removed, weighed, and their volume measured by displacement of saline. They were then fixed for histologic examination by inflation with formalin in saline at a pressure of 1 kPa. Sections of wax-embedded lungs were cut, stained with either hemotoxylin and eosin or Vernhoff's method for collagen, and examined for evidence of fibrosis.

Results

Pattern of Breathing

Control Pattern. The fibrotic animals breathed with a shallower and more rapid pattern than did the control rabbits: control $t_I = 0.56 \pm 0.12$ s; $t_E =$

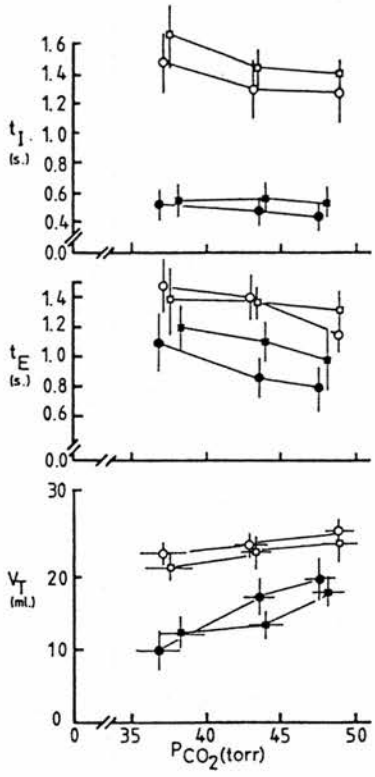

Fig. 2. Effect of increased end-tidal PCO_2 on, from top down, mean inspiratory duration (t_I), expiratory duration (t_E), and tidal volume (V_T) ± 1 S.E.M. in normal (■) and fibrotic (●) rabbits before vagotomy and normal (□) and fibrotic (○) rabbits after vagotomy.

1.19 ± 0.15 s; V_T = 12.1 ± 2.5 ml (n = 16); fibrotic t_I = 0.51 ± 0.10; t_E = 1.10 ± 0.27; V_T = 10.0 ± 3.1 (n.s.) (n = 16 tests in 8 rabbits).

Total lung resistance of the fibrotic rabbits was not significantly higher than the control value (control, 2.85 ± 0.15; fibrotic, 3.0 ± 0.25 kPa/L/s; means and standard errors, 8 tests in 8 rabbits each group, $p < 0.1$). Compliance was reduced in the fibrotic rabbits (control 72.0 ± 5.0, fibrotic 62.0 ± 5.1 ml/kPa; means and standard errors, 8 tests in 8 rabbits each group, $p < 0.1$).

Bilateral cervical vagotomy increased the duration of both t_I and t_E and increased V_T in both the control and fibrotic animals, and reduced the absolute differences between the 2 groups (Fig. 2).

Carbon Dioxide. CO_2 was added to the inspired air of the two groups of rabbits before and after bilateral vagotomy. End-tidal partial pressure was increased in 2 stages from approximately 35 mmHg to 45 and then 55 mmHg (group mean values).

Fig. 2 summarizes the results for the 2 groups of rabbits breathing air and the 2 concentrations of carbon dioxide. In the vagally intact state, both groups of rabbits increased minute ventilation by reducing t_E and increasing V_T. The absolute increase in V_T was greater in the fibrotic than in the control group.

After vagotomy the increase in ventilation and frequency due to CO_2 was smaller but there was still a significant increase in V_T in both groups. The differences between the responses of the control and fibrotic groups were reduced by vagotomy.

Hering-Breuer Inflation Reflex. Inflation of the lungs of 8 control rabbits (13 tests) with a positive pressure of 1 kPa (10 cmH_2O) produced an average pause of 18.9 ± 8.7 times the duration of the previous expiration. In 8 fibrotic animals (13 tests) the pause was significantly ($p < 0.05$) shorter, being only 11.9 ± 2.3 times the duration of the previous expiration.

Steps of Deflation. The effects of steps of deflation of the lungs by a pressure of −1 kPa (−10 cmH_2O) were analyzed for the first and sixth breath after deflation. In 8 control rabbits 20 steps of deflation produced 8 augmented breaths, which were identified as having t_I greater than 1.5 × control and a double peak of inspiratory flow (an average of 2.5 deflations was required to produce an augmented breath, an average of 1 per rabbit). In 8 fibrotic rabbits 20 steps of deflation produced 18 augmented breaths (an average of 1.1 deflations to produce an augmented breath, an average of 2.3 per rabbit). This difference between control and fibrotic rabbits was significant at the $p < 0.01$ level.

The frequency of breathing at the midpoint of the step of deflation, as defined by the characteristics of the 6th breath into the deflation, was also different in the control compared to fibrotic rabbits. This was due to significant ($p < 0.001$) differences in t_E. t_I was not significantly changed from its control value. In the control rabbits t_E fell from 1.10 ± 0.07 to 0.54 s ± 0.03; in the fibrotic rabbits t_E fell from 0.95 s ± 0.04 to 0.46 s ± 0.05.

Pulses of Inflation and Deflation. In 8 control rabbits 20 pulses of inflation provoked 7 augmented breaths. Twenty pulses of deflation produced 2 augmented breaths. Both these figures are significantly less ($p < 0.05$, Chi square test) than those obtained with 8 fibrotic rabbits, in which 20 pulses of inflation produced 16 augmented breaths and 20 pulses of deflation produced 8.

Histologic Examination. Morphologic evidence of lung damage was seen in all the animals studied. There were areas of alveolar collapse and consolidation consisting of subpleural lesions involving either an early diffuse thickening of the alveolar septa or discrete nodules containing fibrous material (Fig. 3). In 1 animal an area of focal emphysema was also seen. Within the larger lesions the epithelium of the conducting airways was thickened, indicating epithelial hyperplasia. Alveolar epithelialization or type II cell proliferation was also evident. The airspaces at the site of the lesions frequently contained large numbers of "foamy" macrophages, which presumably contained the remains of the oleic acid. The lesions appeared to be of various ages. While the collagen staining was variable between sections, all the lungs showed evidence of early fibrosis. Focal hemorrhage was seen in some areas and the walls of the blood vessels within the lesions were frequently thickened (Fig. 3), on occasion to the extent that the lumen was virtually occluded.

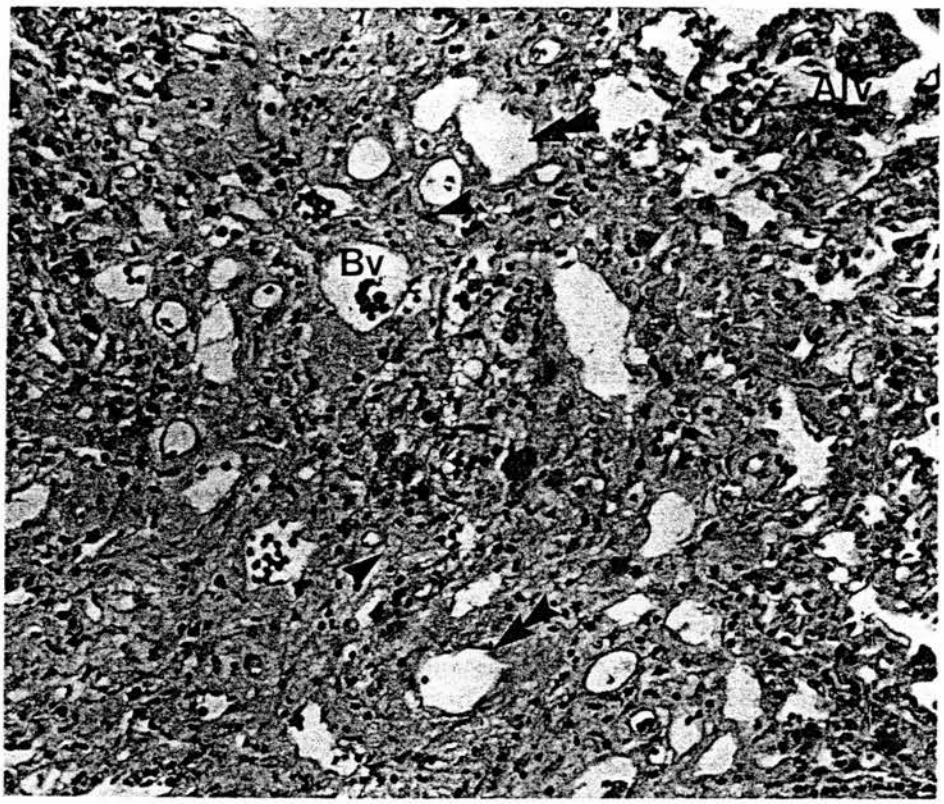

Fig. 3. Photomicrograph of a fibrotic lung of a rabbit shows part of a nodular aggregation of fibrous tissue stained with hemotoxylin and eosin. The lesion is made up of fibrous material, which is probably collagen (*single arrowheads*). Some of the blood vessels (*Bv*) running through the lesion have thickened fibrous walls (*double arrowheads*). An area of collapsed alveoli can be seen at the top right (*Alv*) (×300).

Blood Gases, pH, and Lung Density. pH, Po_2, and Pco_2 were measured in samples of arterial blood taken from the femoral catheter of 5 normal and 5 fibrotic rabbits at the beginning of the experiment. Control values were 7.36 ± 0.037; 91.0 ± 13.7 mmHg, and 41.2 ± 2.39 mmHg, respectively. In the fibrotic rabbits the values were 7.40 ± 0.034, 89.4 ± 7.2 mmHg, and 37.1 ± 1.10 mmHg, respectively. The rabbits had body masses of 3.2 + 0.3 kg (control) and 3.5 ± 0.12 kg (fibrotic). The excised lungs had mean volumes of 23.3 ± 2.4 ml (control) and 26.5 ± 1.3 ml (fibrotic) and weighed 15.5 ± 2.5 and 21.1 ± 2.9, respectively. The fibrosed lungs therefore had a significantly greater specific gravity (0.8 ± 0.7) than those from the control animals (0.6 ± 0.1).

Receptor Activity

The activity of 31 afferent vagal fibers was recorded in control rabbits and 41 in fibrotic rabbits. In the control rabbits 3 fibers were associated with rapidly

Table 1. Action potentials in control and fibrotic rabbits

		Control				Fibrotic			
		Total spikes in	f(S^{-1})			Total spikes in	f(S^{-1})		
			Max	Mean	Min		Max	Mean	Min
Control		46.2	72.0	48.2	24.0	118.00*	126.5*	93.8*	46.9*
breathing	t_I	±16.1	±7.0	±21.1	±7.0	±35.40	±100.8	±17.9	±19.0
(n = 20)	t_E	39.7	48.0	29.1	21.0	58.0*	72.2*	45.9*	31.7*
		±18.1	±18.8	±12.5	±9.6	±15.8	±14.2	±9.8	±10.4
CO_2		45.4	75.6	52.0	26.0	113.9*	154.3*	108.1*	30.6
(55 mmHg)	t_I	±18.7	±3.1	±22.0	±8.0	±34.1	31.0	±25.0	±14.0
(n = 8)	t_E	31.5	51.0	29.0	24.0	33.9*	71.9	34.7	20.0
		±18.8	±23.1	±17.2	±22.0	±16.3	±37.9	±14.3	13.2
Inflation		63.1	94.0	63.1	50.2	138.0*	188.0*	138.0	107.1
(+ 1 k Pa)	1st S.-	±15.0	±17.0	±15.0	±19.2	±35.2	±64.0	±35.2	±55.0
(n = 8)	2nd S.-	58.2	70.2	58.2	47.1	113.0*	140.2*	113.0*	100.0
		±15.0	±19.6	±15.0	±19.0	±35.2	±48.0	±25.3	±45.0
	3rd S.-	55.2	64.5	55.2	53.0	112.1*	132.4*	112.1*	105.1
		±16.0	±15.6	±16.0	±15.7	±26.6	±40.8	±26.6	±54.4 (n.s)
Deflation	1st breath	32.8	46.2	28.0	35.0	89.9*	92.4*	63.0*	32.2
(−1 k Pa)	t_I	±14.7	±16.6	±11.8	±14.4	±17.0	±33.6	±17.6	±13.0
(n = 8)		9.0	36.0	20.2	15.3	34.7*	86.3*	58.0*	42.0*
	t_E	±3.0	±13.4	±6.4	±8.0	±16.0	±3.5	±22.4	±16.5
	6th breath	20.1	50.2	30.0	23.2	65.6*	106.3*	73.6	45.1*
	t_I	±8.9	±20.4	±12.8	±7.0	±28.5	±44.8	±24.7	±17.0
		13.6	36.0	21.0	20.1	29.3*	76.8*	56.6*	47.6*
	t_E	±7.57	±14.6	±6.4	±6.6	±8.2	±28.2	±16.1	±16.0

The number of action potentials in vagal "single fibers" associated with slowly adapting pulmonary receptors in the lungs of eight control and eight fibrotic rabbits during (n) tests is shown (± S.E.M.) Total counts in inspiration (t_I) and expiration (t_E) and the maximum mean and minimum discharge rate in both phases are given during eupnea and CO_2 inhalation. During the breathing pause caused by inflation of the lungs, activity during the first consecutive 3s is given. During lung deflation, activity in the first and sixth breath is given. Significant differences ($p < 0.05$) between the control and fibrotic rabbits are shown (*)

adapting receptors (RAR) and in the fibrotic animals 11 receptors were of this type, a difference significant at the $p < 0.01$ level (Chi square test).

Activity of pulmonary stretch receptors was quantified (Table 1) under the conditions described in the Pattern of Breathing section above, for the 2 phases of each breath, in terms of the total number of action potentials occurring in each phase: the maximum, minimum, and mean frequency of discharge (±S.D.) for each of n breaths sampled from 8 rabbits. Student's t-test for uncorrelated means was used to compare the significance of the difference between the means of the control and fibrotic rabbits. No significant difference (n.s.) was taken to be a probability >0.05 that the changes observed were due to chance.

During carbon dioxide accelerated breathing the rabbits were unilaterally vagotomized to allow recording of receptor activity. The pattern of breathing was therefore not comparable with the intact or bilaterally vagotomized states.

During steps of inflation (1 kPa), because the Hering-Breuer reflex arrested breathing, analysis in terms of t_I and t_E would have been meaningless. Therefore, the activity in the first 3 consecutive seconds of inflation was analyzed and is shown in Table 1. Steps of deflation were maintained for 8 breaths and stretch receptor activity during the 1st and 6th breath of deflation is shown in Table 1.

Discussion

Patients with pulmonary fibrosis usually present with reduced pulmonary compliance and an increased minute ventilation and breathing frequency that are not chemically mediated [16]. In advanced stages they often complain of severe dyspnea. It has been accepted for many years that vagal activity from the lungs affects the pattern of breathing, at least in animals, and that sensations from the lungs are probably transmitted by this route [11]. It seems reasonable to expect, therefore, that the patterns of breathing and sensations of dyspnea suffered by patients with lung disease may be influenced by activity from pulmonary receptors.

Rather than blocking [11, 23, 28] or recording bulk vagal activity [1, 10], as other workers have done in disease models produced by acute injury, we have recorded the type and degree of activity of individual receptors. Such an analysis is relevant to the cyclic change in sensitivity to slowly adapting [6] and rapidly adapting [7] receptors at different times in the respiratory cycle.

A prerequisite for the present study was a valid model of human disease. Although animal models of human lung disease do not exactly reproduce what is seen in humans, "the usefulness of an experimental model should be judged on how well it answers the specific questions it is being used to answer rather than on how well it mimics human disease" [27]. To mimic the histologic changes seen in human fibrosis, we adopted the slowly developing model of pulmonary interstitial fibrosis produced by Derks and Jacobovitz-Derks [8]. In this preparation, repeated intravenous injections of oleic acid resulted in neutral fat pulmonary embolism followed by a chemical stage related to the toxicity of the free fatty acids liberated by the action of pulmonary lipase on the neutral fat [22]. This resulted in a fibrosis model that fulfilled Carrington's [4] criteria of: mixed cellular exudate in interstitium, proliferation of the lining epithelium, protein exudate in the airspaces with leukocytes in alveolar spaces, gradual progression to fibrosis, and diffuse distribution.

Our fibrotic rabbits showed the reduction in compliance seen in human interstitial lung disease. Breathing patterns reported for human patients with lung fibrosis are varied, ranging from relatively normal [17] to the more generally reported rapid shallow pattern, which is not chemically mediated [24]. Our rabbits conformed to this rapid shallow pattern both at rest and when breathing was accelerated by CO_2 (Fig. 2). Under these circumstances, which were induced to mimic breathing during exercise, which is when fibrotic patients suffer the most disability due to dyspnea, changes in frequency and tidal volume

appeared greater in the fibrotic animals, although this difference did not reach a level of statistical significance. The fact that reflex changes produced by inflation or deflation of the lung produced more profound and statistically significant differences between the control and fibrotic rabbits emphasizes the possible importance of reflex activity in the production of dyspnea in fibrotic patients on exercise, when increased minute ventilation would be more likely to provoke such reflexes than at rest.

The response to CO_2 persisted after vagotomy. Phillipson and co-workers, [23] exercised conscious dogs with pulmonary fibrosis. They found, as we did, that changes in pattern of breathing in response to their stimulus survived total vagal block and were greater in the fibrotic than the normal dogs. This could indicate that extravagal factors, such as receptors in the chest wall [9], may have been responding to the added elastic load of breathing from reduced lung compliance.

The Hering-Breuer inflation reflex is essentially a prolongation of t_E mediated by pulmonary stretch receptors [14], 60% of which are active at end-expiratory volumes [21]. The duration of the Hering-Breuer pause was significantly shorter in the fibrotic than in the control rabbits, despite stretch receptors being more active in the fibrotic lungs. Some t_E-shortening inspiratory drive must have been active in the fibrotic rabbits. This drive was most probably vagal, since vagotomy abolished the Hering-Breuer reflex in both cases and inflation did not then accelerate breathing. The most likely inspiratory drive was rapidly adapting receptors, with some unquantified contribution from unmyelinated fibers.

Lung deflation by negative pressure or pneumothorax diminishes stretch receptor activity [13] and increases rapidly adapting receptor activity [7, 12, 26]. J receptors are only slightly stimulated, however, and then only in extreme degrees of lung collapse [21]. These changes in activity are confirmed by our results and reflected in the pattern of breathing. In particular, rapidly adapting receptor activity causes an augmentation of the first breath during deflation. The frequency of augmented breaths depends on the intensity of rapidly adapting receptor activity and its position in a refractory period [7] that can last for several minutes. In the present series of experiments a significantly greater number of augmented breaths occurred during deflation of the fibrotic rabbits' lungs than in the control rabbits. This suggests greater than normal rapidly adapting receptor activity. This was borne out by direct recording. Slowly adapting pulmonary receptor activity, illustrated in Fig. 1, is almost identical in magnitude in the control rabbits to that recorded in an earlier series of experiments [7]. Peak frequencies of discharge are lower than those obtained from receptors in the extrathoracic trachea [25], which can be explained by differences in compliance of the 2 preparations. Activity of slowly adapting receptors was increased by fibrosis, during both eupnea and inflation by positive pressure. This would not be expected from the observed reduction in the Hering-Breuer reflex in the fibrotic rabbits, and was presumably due to some other vagal afferent activity, probably from RAR exerting a Sheringtonian "prepotent" inspiratory drive. Another stimulus in the fibrotic animals may have been a

more rapid development of hypoxemia and hypercapnia owing to the smaller lung volume during inflation than in the controls.

The number of RAR (3) found in the normal rabbits was of the order expected from other studies [7]. From the same study it is known that increased RAR activity shortens t_E and promotes augmented breaths. A highly significantly greater number (11) of RAR were found in the diseased rabbits and were probably the origin of the highly significantly greater number of augmented breaths recorded in these rabbits. It is, of course, unlikely that fibrosis would increase the number of any type of receptor. The number of slowly adapting receptors may, in fact, have been reduced, altering their ratio to RAR. It is also possible that a number of RAR, silent during eupnea in healthy lungs, would become active when fibrosis was induced. The present study provides no information about the activity in unmyelinated fibers in the fibrotic lungs of rabbits, and we suggest that this work be repeated in cats, in which C-fiber activity can be recorded more easily.

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The effect of high-frequency ventilation on non-Newtonian properties of bronchial mucus

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We have investigated the changes in the non-Newtonian properties of human bronchial mucus brought about by *in vitro* high frequency ventilation. This type of ventilation brought about changes in viscous properties, measured during creep and oscillation of the mucus, which would be expected to reduce mucus clearance *in vivo*. We suggest that any beneficial effects of clinical high-frequency ventilation on respiratory mucus clearance in patients are not brought about by long-term (more than a few seconds) changes in the viscous properties of the mucus itself.

Introduction

Interest has recently been focused on the possibility of increasing respiratory mucus clearance in diseases such as cystic fibrosis and chronic bronchitis by vibrating the air in the airways at a frequency of about 10–20 Hz and a tidal volume comparable to anatomical dead space (High Frequency Ventilation, HFV). Some *in vivo* animal and human experiments have suggested that clearance may be improved (1–4), while others have failed to demonstrate an improvement (5–7).

If HFV alters mucus clearance the effect could depend on a number of factors including short and long-term effects on the rheology of the mucus, alterations in the volume of the mucus or the sub-mucus phase, and changes in the function of the respiratory tract cilia. Of these, changes in the rheology of the mucus seem very likely since mucus has been shown to have shear thinning properties (8).

The rheology of mucus during HFV has not been extensively studied *in vitro*. King *et al.* (9) reported a decrease in viscosity with oscillation, while Hachenberg *et al.* (5) noted an increase. A shortcoming of some previous investigations has been the use of methods of measuring viscosity which, though suitable for Newtonian liquids, are inappropriate for highly non-Newtonian mucus. Investigators have measured changes in viscosity of expectorated sputum. This consists of saliva plus bronchial secretion which itself is made up of tissue fluid transudate and, from goblet and serous mucus cells, the acid glycoproteins which make up the bronchial mucus which gives sputum its characteristic non-Newtonian properties.

We have used expectorated sputum in the present series of experiments because of its availability and because it contains the actual substance whose clearance one would hope to improve in clinical situations. Other workers have used 'pure' mucus obtained from, for example, tracheal secretions; whilst this is more easily-defined in chemical terms, it does not accurately represent the substance expectorated by patients in the clinical situation. Although in these experiments the term 'High Frequency Ventilation' is slightly inappropriate, no lung or equivalent ventilated structure being involved, we use the term as we believe the effect of the oscillations produced in the sputum to be akin to those produced by HFV; and to differentiate the experimental treatment from the subsequent oscillatory measurements made in the rheometer.

In the present investigation we subjected expectorated human bronchitic sputum to *in vitro* high frequency ventilation for various periods of time. The effect on its rheology was measured using a parallel plate rheometer and procedures suitable for non-Newtonian liquids. These procedures were steady shear flow, creep, and small amplitude oscillatory shear flow. Oscillation determines the elastic as well as the viscous response, whilst creep gives some indication of the relaxation processes in the fluid. Our measurements of viscosity were made following periods of simulated HFV, as in the clinical situation expectoration would normally follow HFV treatment. Our results suggest that HFV causes a slight increase in both the viscosity and elasticity of the sputum. Any

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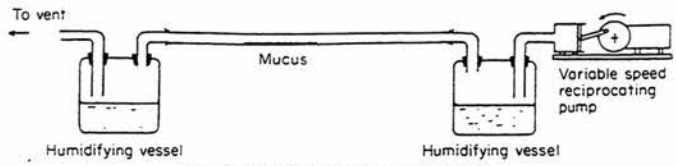

Fig. 1 The high frequency ventilator.

improvement in clearance resulting from HFV is therefore probably brought about by a mechanism other than reduction in sputum viscosity.

Methods

Sputum expectorated overnight was collected from eight inpatients in the respiratory ward of Edinburgh City Hospital. These patients were all suffering from infective exacerbation of chronic obstructive airways disease at the time of sputum collection. The individual sputum samples were kept in closed containers in contact with water saturated air and rapidly transferred from container to rheometer or high frequency ventilator to prevent drying. In each case sputum from one patient was used for each complete set of measurements, i.e. each sample acted as its own control. From each sample 5-ml aliquots were taken and subjected to 0, 1, 2 and 4 min of high frequency ventilation designed to mimic as closely as possible the pattern of ventilation which has been used to investigate the effect of HFV on human mucus clearance. The aliquot was placed in a soft vinyl tube 400 mm long with a 10 mm internal diameter. The tube curved down slightly to retain the mucus. Both ends of the tube were connected to water seals to maintain 100% air saturation (Fig. 1). The air in the tube was oscillated through one of the water-seals by a reciprocating piston pump to imitate *in vivo* HFV. The frequency of oscillation was 10 Hz and the displacement 100 ml. Air temperature was accurately controlled at 20°C. This gave rise to maximum pressure changes in the tube of less than 10 Pa (compared to normal air pressure of 10^5 Pa). At this stress level, it will be shown later, the maximum shear rate generated is about $1{\cdot}0\ s^{-1}$. The aliquot was then rapidly transferred to a Carri-Med Controlled Stress Parallel Plate Rheometer (Carri-Med Ltd, Dorking, U.K.) where its creep, oscillatory and flow properties were measured at 37°C between plates of 5 cm diameter separated by a gap of 100 μm. This instrument can resolve shear rates as low as $10^{-6}\ s^{-1}$ over displacements as low as 10^{-6} radian. Strain amplitudes during oscillations can be as low as 5×10^{-3}. These low levels of sample deformation are necessary in investigations of non-Newtonian materials and have not been achieved in many other investigations.

To control the small water loss that might have taken place through the 100-μm gap between the rheometer plates they were surrounded by a water-controlled dam which ensured 100% water saturation of the surrounding air.

The procedure was repeated at least three times, for each duration of HFV using different aliquots of the same sample, and results averaged for the aliquots in each time group.

Measurement of creep properties were made by applying a single-small stress to the sample and monitoring the resulting strain (deformation) which takes place with time. The strain divided by the stress is the creep compliance, which is the reciprocal of elasticity. The stress was chosen to exceed the elastic limit and produced the characteristic yield point curve of visco-elastic substance. After 60 s, the stress was removed and the strain experienced by the sample during relaxation was recorded. Measurement of oscillatory properties were made by subjecting each sample to a sinusoidal torque of 10^{-5} N.m. over a very small amplitude of rotation (10 m radians) which would not cause sample breakdown, over a range of frequencies from 1 to 8 Hz. The resultant amplitude and phase shift were recorded at each frequency, from which the dynamic viscosity and elastic modulus were calculated. To measure flow properties, the shear rate generated in the sample was measured as a function of an increasing applied shear stress. The viscosity was determined from the stress divided by the shear rate. Because stress and not the speed of flow of the material was controlled, the viscosity at different flow rates were calculated together with a yield point at which flow began.

Results were obtained in numerical and graphical form (Figs 2–4, Table 1) for aliquots exposed to 0, 1, 2 and 4 min of HFV. Viscosity was compared for each of the aliquots oscillated at 1, 2, 4 and 8 Hz in the rheometer and during increasing strain and stress during creep and flow.

Significance of changes produced by HFV was tested by the Chi-squared test. Probabilities $<0{\cdot}05$ were considered significant.

Table 1 Results of measurement of dynamic viscosity η' and storage modulus (G') of a typical sputum sample before and after high frequency ventilation for 0, 1, 2 and 4 min. The measurements were made at 1, 2, 4 and 8 Hz oscillation in the rheometer at 37°C

Rheometer frequency (Hz)	Minutes of HFV			
	0	1	2	4
Dynamic viscosity, η' (Pa.s)				
1	0·1470±0·0191	0·1972±0·0468	0·3071±0·0342	0·3824±0·0183
2	0·0805±0·0137	0·1134±0·533	0·1653±0·0259	0·1935±0·0317
4	0·0511±0·0091	0·0697±0·0254	0·0912±0·0127	0·1048±0·0213
8	0·0375±0·0063	0·0483±0·0118	0·0598±0·0088	0·0654±0·0120
Storage modulus, G' (Pa)				
1	2·006±0·628	2·436±0·582	4·075±0·618	4·063±0·578
2	2·106±0·523	2·694±0·716	4·228±0·593	4·160±0·472
4	2·246±0·392	2·874±0·431	4·484±0·368	4·365±0·319
8	2·186±0·278	2·693±0·268	4·252±0·237	4·413±0·217

Sample taken on 22 January 1990.

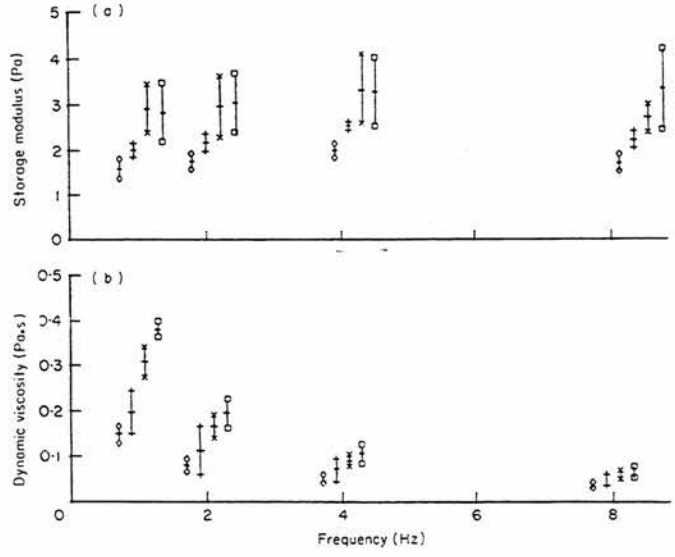

Fig. 2 Storage (elastic) modulus (a) and dynamic viscosity (b) of eight samples of sputum measured at oscillations of 1, 2, 4 and 8 Hz after HFV for: (◇) 0; (+) 1; (X) 2; and (□) 4 min. The bars represent 1 SE either side of the mean and have been spread for clarity.

Results

OSCILLATORY TESTS

Table 1 contains the results of measurements on a typical sample of sputum, each of which acted as its own control before and after HFV for 1, 2 and 4 min. Dynamic viscosity (n') and storage modulus (G') were measured at 1, 2, 4 and 8 Hz and were significantly increased ($P<0·05$) for the samples ventilated for 1 and 2 min, after which time little further increase was noted. Decrease in dynamic viscosity with increase in rheometer oscillating frequency (1, 2, 4 and 8 Hz) demonstrates that the samples used were truly visco-elastic. Figure 2 shows the dynamic viscosity and the

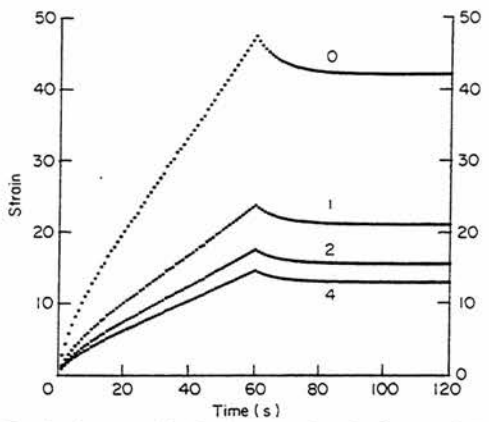

Fig. 3 Creep test. Strain plotted against time for a typical sample of sputum before and after 1, 2 and 4 min HFV. The number of HFV minutes is indicated for each curve. The imposed stress was 10 Pa applied for 60 s. The sample was taken on 24 January 1990.

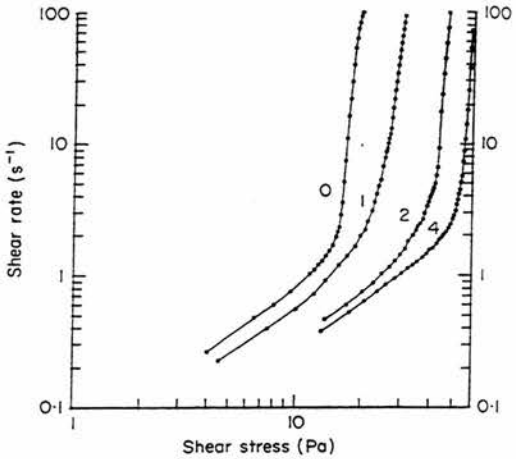

Fig. 4 Flow test. Shear rate plotted against shear stress for a typical sample of sputum before and after 1, 2 and 4 min HFV. The number of HFV minutes are indicated for each curve. Sample taken on 25 January 1990.

storage modulus, as functions of the imposed frequency, the values being pooled results of all samples. Movement of these curves away from the abscissa with increasing time of ventilation indicates a thickening of the sample. Though there was inter-sample variation, all intra-sample results were qualitatively similar.

STRAIN TESTS (CREEP)

Figure 3 shows a typical result of the measurements on individual samples of sputum, each of which acted as its own control before and after high frequency ventilation for 1, 2 and 4 min. Results from all samples showed the change in position of the curve with increasing duration of ventilation seen in this example. Strain is plotted against time and the curve produced is typical of visco-elastic liquids. As long as the sample remains visco-elastic the curve will remain the same shape, as is seen in Fig. 3. The initial rapidly rising phase corresponds to the initial elastic response of the sample to the (extremely small) applied stress. Over all samples this was found to be $1{\cdot}10 \pm 0{\cdot}35$ units. The linear region approaching 60 s is associated with purely viscous behaviour, the slope of the curve being the shear rate of the flow. Since the imposed stress was the same for each experiment, the reciprocal of the slope is proportional to the viscosity. An increase in viscosity of the sample would therefore be associated with a curve closer to the abscissa. The curved section which links the viscous and elastic portions of the curves is referred to as the retarded elastic region and typifies visco-elastic behaviour. It can therefore only be quantified in terms of viscosity and elasticity. The curves were fitted to a Kelvin model with two retardation times. These did not change significantly with duration of ventilation, or between samples, the values being 12 ± 2 s and $1{\cdot}75 \pm 0{\cdot}45$ s. Similarly, the relaxation curves were fitted to a Kelvin model with two relaxation times which were found to be $20{\cdot}3 \pm 2{\cdot}2$ s and $2{\cdot}15 \pm 0{\cdot}75$ s. Again, these did not change significantly with duration of HFV, or with different samples.

FLOW TESTS

In conventional flow measurements, using capillary falling sphere or efflux viscometers, high shear rates are developed which destroy the structure of mucus (10) in such a way as to prevent any meaningful measurements of elasticity being made. For completeness, however, we include a plot of shear rate against shear stress (Fig. 4) for a sample ventilated for 0, 1, 2 and 4 min. The phase of increasing stress has been plotted and shows the typical shape of flow curve obtained by other workers, with viscosity falling from an initial, high valued, plateau, to relatively low values at high shear rates. Once again, the overall value of the viscosity has been increased by the initial high frequency ventilation.

Discussion

It was our aim to reproduce, as closely as possible, the conditions under which mucus would be treated

during high frequency oscillatory ventilation. The collected mucus was therefore kept in closed containers in contact with water-saturated air at all times. It was subjected to HFV in a tube with a diameter of the order of a human main bronchus which was protected at both ends by water seals to maintain 100% saturation. Water aerosols have been used by investigators to maintain water saturation of their samples. These aerosols most probably contaminated the mucus and contributed to the reported fall in viscosity. Manipulation of the mucus in our experiments was kept to an unavoidable minimum.

Such manipulation inevitably changes mucus rheology and we can only say that the control and test samples received as near as possible the same treatment. HFV did not introduce visible bubbles of air into the mucus. Other workers (5) centrifuged their samples to remove air which would affect the method of measurement they used. We considered that any such air trapping (which could not be seen in our samples) would equally occur *in vivo* and should not be interfered with.

The study of flow and deformation behaviour of respiratory tract mucus is made difficult by the fact that it is a delicate non-Newtonian material whose properties are radically altered by attempts to measure them by methods more suitable for Newtonian liquids. Such methods include measuring flow through orifices or capillary tubes, and are unsuitable for fluids such as mucus in that the high shear stresses inevitably generated destroy any structure present before any meaningful measurements can be taken. The instrument used in the present study was a parallel plate rheometer specifically designed for the non-destructive measurement of the properties of non-Newtonian heterogeneous material. After such non-destructive testing our samples were finally subjected to the more conventional Flow Test used by a number of other workers which alters the properties of the mucus and therefore renders it unsuitable for further investigation (10). The three types of investigation applied to the samples can be considered independently.

OSCILLATION

This is a non-destructive technique for investigating the structure of delicate materials over short and medium periods of time. In this respect it partners the creep mode (see later) which provides visco-elastic data over longer periods. The technique involved subjects the sample to a sinusoidal stress or strain wave of amplitude small enough to be non-destructive. If the material was purely elastic then the stress and strain waves would have been in phase since stress is proportional to strain (Hookes Law). If the sample was purely viscous then the stress and strain waves would have been 90° out of phase since for a Newtonian liquid, stress is proportional to strain rate. The phase shift in our samples varied between 25° and 53°. A highly significant number ($P<0{\cdot}01$) of our samples showed an increase in viscosity and elasticity as a result of HFV.

The samples were subjected to oscillation at 1, 2, 4 and 8 Hz. A fall in dynamic viscosity with increased frequency of oscillation demonstrated the samples were visco-elastic.

CREEP

The levelling of uneven layers of mucus due to surface tension or sagging due to gravity are stress controlled situations. Measurements of creep properties of mucus tell us how it will behave under these conditions. In this part of the investigation a single small stress was applied to the sample and the resulting strain (deformation) monitored. In Newtonian fluids constant stress will produce a constant shear rate and any strain that has occurred will not be recoverable once the stress is removed. In visco-elastic materials the application of stress will give a characteristic creep curve. This will reach equilibrium, below the elastic limit, after which no further flow occurs and any strain is completely recoverable, or reach an equilibrium shear rate beyond the elastic limit. Treatment of our samples with HFV resulted in a decrease in strain values, and hence shear rate, for the constant stress applied. HFV therefore seems to produce a more viscous sputum.

FLOW

Because, as its name implies, this measurement produces significant movement in the material of the sample it changes the properties of non-Newtonian substances such as mucus. For this reason this measurement was carried out last in each series. In this test the variation in viscosity of the sample was measured as a function of shear stress. Measurements of flow related viscosity predict how mucus will behave when forced to flow over a surface or through an orifice. Because stress and not speed of movement was controlled in this test it was possible to determine whether the samples exhibited a yield value (11). With the alternative controlled shear rate type of instruments, each shear rate must produce a corresponding finite shear stress, and yield values can only be determined by extrapolation from the data. Our samples did not exhibit yield stresses. Once again, HFV increased the viscosity, the increase varying directly with the duration of ventilation. The viscosity

curve mirrors that obtained on controlled shear rate instruments by Sturgess *et al.* (12) for human sputum.

The application of our findings to the clinical situation relates, of course, to only one aspect of clearance, albeit a very important one. Ciliary activity will undoubtedly be inhibited by an increase in viscosity and the stickiness of tack of mucus must be of importance in clinical clearance. Stickiness or tack is a rheological property dominated by the elongational viscosity of the fluid involved (13). Although such studies were not conducted on our samples, and are indeed extremely difficult to perform, results from fluids of similar viscoelastic properties suggest that decreasing the shear viscosity of the fluid, at a particular level of shear rate, also decreases the elongational viscosity at an equivalent strain rate, and hence reduces the tac. The reverse is also true. Our experimental set-up is quite different from that used by King *et al.* (9). The chief difference is that King made measurements during HFV while we made measurements after HFV the time, under clinical conditions, when expectoration would be made. It is quite likely that mucus has time-dependent (thixotropic) properties. If after a period of ventilation the mucus is relaxing to some rest state, then the tests performed were 'tapping in' on this process. As the time between the end of HFV and the start of each test was roughly the same, the differences in viscosity and elasticity of the sample at that time reflect the visco-elasticity of the sample at the end of HFV. Although our results do not support the suggestion that HFV reduces viscosity of respiratory mucus they do not exclude the possibility that mucus clearance *in vivo* may be improved by other mechanisms during HFV. These include changes in mucus production and rate of ciliary beating within the respiratory tract. The potential advantages of HFV over the rigors of daily physiotherapy for patients suffering retained secretions are such that the technique should continue to be vigorously investigated.

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A COMPARISON OF THE MECHANOGRAM OF THE ANKLE JERK IN MEN AND WOMEN: OBSERVATIONS USING AN ADJUSTABLE DORSIFLEXING TORQUE, HIGH INERTIA MECHANICAL FILTER AND AUTOMATIC READOUT SYSTEM

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SUMMARY

An instrument of new design has been constructed to measure the contractions of the calf musculature resulting from a tap on the Achilles tendon. The instrument provides a predetermined and adjustable dorsiflexing torque from a printed motor, but the contractions are virtually isometric as the system is one of high inertia. Peak force, half-contraction time and half-relaxation time are monitored by electronic circuits equipped with digital output meters. Observations have been made on fifty-two male and forty-five female medical students. There were no significant differences in the peak torques generated by the contractions between the men and women students and no difference in the half-contraction times. The half-relaxation times of the women were, however, significantly longer than those of the men ($P = 0{\cdot}0001$). In another group of students the EMG discharges following a tendon tap were recorded, there was no significant difference in the duration of the activity in men and women. Observations have also been made on twenty-three male and seventy female subjects whose mean ages were in the mid-sixties. The peak torques generated by the contractions were significantly higher in the women ($P = 0{\cdot}03$). There was no difference in the half-contraction times, but the half-relaxation times of the women were significantly longer ($P = 0{\cdot}001$). Possible reasons for the differences are discussed.

INTRODUCTION

As has been established many times, a tendon jerk is characterized by a single, essentially synchronous, burst of activity in the motor units involved. It has accordingly been apparent to a number of investigators that observations of the mechanical events following a tap to a tendon offer the possibility of obtaining rapidly and non-invasively some information about muscle properties. Measurements of the contraction of the calf musculature have been used to investigate thyroid function by many investigators. The relaxation time increases in hypothyroidism and decreases in hyperthyroidism (Chaney, 1924; Lambert, Underdahl, Beckett & Mederos, 1951; Sherman, Goldberg & Larson, 1963; Nuttall & Doe, 1964; Abraham, Atkinson & Roscoe, 1966; Marsden, Meadows & Lange, 1970). Such measurements have also been made, in subjects with diabetes (Beardwood & Schumacher, 1964), obesity (Burt & Stunkard, 1964), cerebral palsy (Wall, Umlauf & Geppert, 1964), on cooling of the calf (Petajan & Watts, 1962) and following the administration of propanolol (Waal-Manning, 1969).

The principal purpose of this paper is to report the use of a new instrument to obtain

information about the range of variation in normal people of peak torque, speed of contraction and speed of relaxation of the ankle jerk. In particular, certain differences have come to light between the sexes both in young and in elderly adults.

METHOD

In the initial set of observations the person sat with the ball of the foot on a 100 kg load cell (RS Components Ltd, PO Box 99, Corby, Northants). A chart recorder was used to obtain graphic records which were then measured. The system was thus isometric but the initial load on the

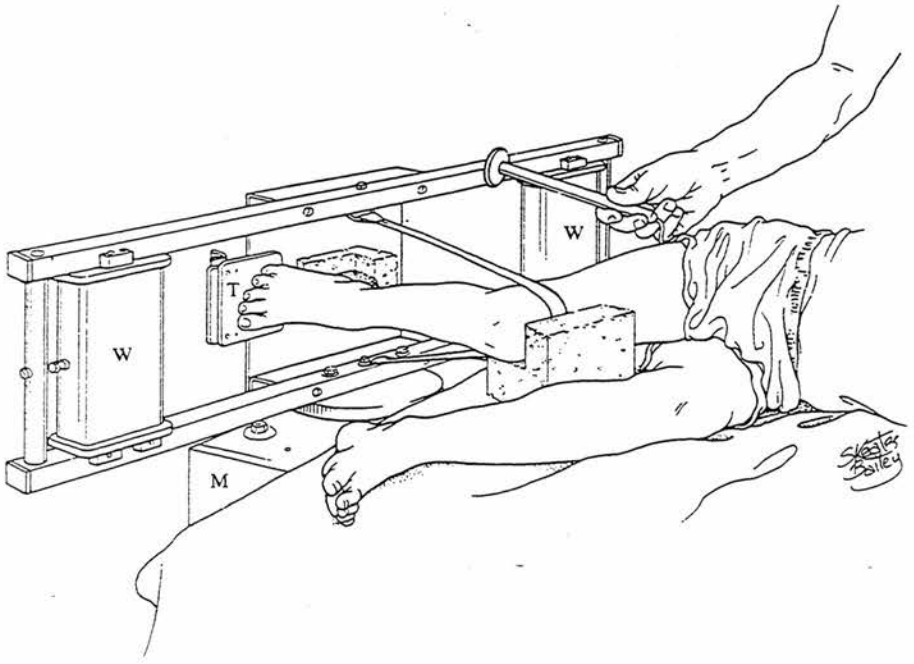

Fig. 1. The apparatus used. The instrument is designed for movements of the foot in the horizontal plane to avoid the confusion which would otherwise result from the effects of gravity. 'M' is the motor housing, 'W' indicates the rectangular canisters into which lead has been cast. 'T' is the plate fixed to the transducer (load cell). Reproduced from Walsh (1992).

transducer varied considerably with the exact placement of the foot and the degree of relaxation. Thus the baseline from which measurements had to be made varied and to keep within the dynamic range of the instrument repeated electrical rebalancing was required. It may be that such problems account for the rarity with which observations have been made isometrically (see Discussion below).

Because of these limitations it was decided to develop new apparatus and this incorporated a large printed motor (G19M4) with a double-ended shaft. The person lay on his side with the knee bent at a right angle. The foot was in contact with a 20 kg load cell (RS Components) mounted on a horizontal beam of length 1·0 m supported at its centre by the upper end of the shaft of the motor. (Fig. 1). The load cell acted as a transducer to register the torque generated by the reflex contraction. The ankle joint was concentric with the axis of the motor. A DC current of up to 10 A was passed through the motor which had a torque constant of 0·28 N m A^{-1}. The initial load on the transducer (load cell) was thus predetermined. Near the ends of the beam were 13 kg lead weights so that the system had high inertia. The brief forces of the jerk thus affected the transducer but were almost over before the beam moved significantly. The apparatus can be regarded as a mechanical filter. The lower end of the shaft of the motor was coupled to a potentiometer recording angular position. By

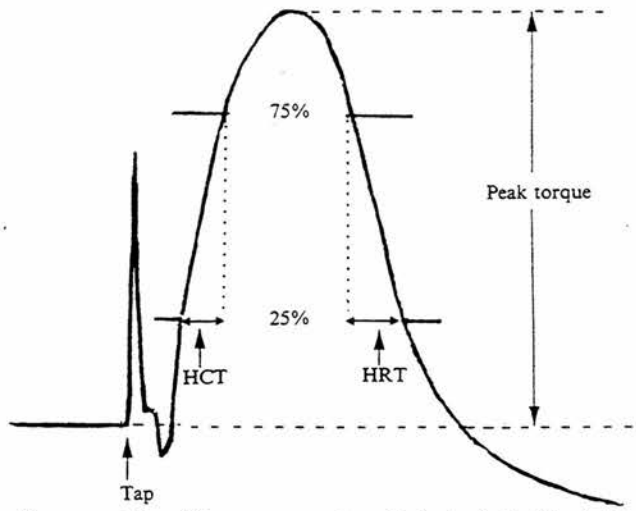

Fig. 2. Diagrammatic representation of the measurements made electronically. The first increase of tension is due to the hammer blow itself. It is brief and is blanked off from the peak detector and timing apparatus. The circuits first detect the peak torque of the contraction; the timing as the torque falls from the 75% to the 25% value is then measured. This is the half-relaxation time (HRT). Finally the signal which has been delayed is fed to circuits which measure the time for the value to rise from the 25% to the 75% value, this is the half-contraction time (HCT). The torque eventually falls below the baseline as the contraction has faded and the beam has reached its highest, albeit low, velocity.

measuring the acceleration resulting from the application of a known torque the inertia of the system was estimated to be 3·1 kg m^2. Measurement confirmed the prediction; the motion might for instance be only 0·035 rad (2 deg) by the time the relaxation was 90% complete. The deflection of the transducer when fully loaded with 20 kg (more than twice the force generated in any of the subjects) corresponded to only 0·003 rad (0·17 deg). By virtue of these arrangements the contractions were virtually isometric.

The output of the transducer was fed to a peak detector and the resultant voltage, corresponding to the maximum torque generated by the contraction, was displayed on a digital voltmeter. The output of the peak detector was also fed to a voltage divider with two comparators, one set to change state as the instantaneous transducer signal fell below the 75% level, the other to change when the output fell under the 25% level. As the voltage fell below the 75% level a gate was opened allowing 1 ms pulses from a crystal oscillator to reach the corresponding liquid crystal counter. When the voltage fell below the 25% level the another gate, in series with the first, closed. The transducer signal was also delayed by 0·3 s and passed to further comparator and timing circuits so that the time taken for the signal to rise from 25 to 75% of the output of the peak detector could also be displayed digitally (Fig. 2). For this arrangement a gate to a second counter was opened as the voltage rose above the 25% level and another gate, in series with the first, was closed when the 75% value was crossed. The circuits were reset automatically by a switch in the tendon hammer. The transducer signal was blanked out for 25 ms following the blow to eliminate confusion from passive vibrations following the tap before the reflex contraction. The voltage divider resistance network giving the 75 and 25% levels, referred to above, was referenced not to earth but to the output of a sample-and-hold circuit. This was activated when the switch in the tendon hammer closed and sampled the output of the transducer which had been fed to the delay line. The 75 and 25% levels were thus the relationships between the peak torque and the load on the transducer just before the blow was delivered.

During the jerk many motor units are likely to become active and, to the extent that different muscle fibres may have different properties, the measurements of timing, made in the middle halves of the contraction and relaxation, will reduce confusion in the results due to such differences. As the measurements have been made when the changes of tension are most rapid the values are shorter than those which would have been obtained had attempts been made to use the first discernible rise of

tension, the crest of the tension and the last detectable point of the contraction. Lambert *et al.* (1951) had noted that 'for accurate measurement... neither the time of the peak of contraction nor the end of relaxation was a sharp end-point...'. The decay of tension from the peak value is not an exponential fall for the curve is sigmoidal in shape.

The knee, which was bent at a right angle, was prevented from moving during the contraction by the use of 'Velcro' strapping. At the start of a series of observations the current through the motor was switched on and was continuous. The use of printed motors in investigating the postural system has been discussed at length in a recent monograph (Walsh, 1992). The motor was obtained from Printed Motors Ltd, Bordon, Hants. Such a device converts an electrical current into the corresponding torque. The force on the ball of the foot due to the energization of the motor took up the slack in the Achilles tendon. If the current through the motor was increased the resting position of the foot became more dorsiflexed. The person was induced to relax, and if relaxation was incomplete the observations were discarded. The tendon taps were deemed to be a maximal stimulus. Further increase in the strength of the blows did not increase the force of the reflex contractions. Where the hammer did not hit the tendon squarely the observations were discarded.

Where reliable contractions were not at first obtained the person was instructed to reinforce the response by squeezing a dynamometer with the right hand. This method of reinforcement was effective; the traditional Jendrassik method could not be satisfactorily used with the person lying on his side. A wide variety of manoeuvres reinforce tendon jerks; some of the procedures were reviewed by Wartenberg (1945).

Fifty-two male and forty-five female medical students were tested, (mean age, $19{\cdot}9 \pm 2{\cdot}4$ years). Four sets of measurements were made on each person. Four amperes of current were passed through the motor except where otherwise stated. This provided 1·12 N m of dorsiflexing torque. To ascertain how sensitive the measurements were to the value of the dorsiflexing torque some observations were made with the current reduced to 1 A giving a value of 0·28 N m.

Twenty-three male and seventy female older subjects were also tested. The mean ages did not differ significantly between men and women, that for the men being $68{\cdot}3 \pm 4{\cdot}8$ years, that for the women $66{\cdot}6 \pm 5{\cdot}0$. The dorsiflexing torque used is documented below. In addition to the apparatus described above, in these observations a chart recorder ran for a short period after the switch in the tendon hammer was activated and the changes in tension recorded from the output of the delay line were displayed. The shape of the tension curve was thus monitored throughout the contraction, the start of the recording being effectively before the tap had been delivered. One subject was rejected from the series as a tendon tap gave rise to a generalized myoclonic disturbance. In this group of subjects certain anthropometric measurements were also undertaken. The lengths of the legs were estimated from measurements of the sitting and standing heights.

Observations were made also on the EMG in response to a tap on the tendon in twenty male science students (mean age, $19{\cdot}1 \pm 0{\cdot}6$ years) and in fifteen female science students (mean age, $19{\cdot}6 \pm 1{\cdot}1$ years). In these observations the jerk was elicited as in a clinical examination the person sitting on a bench, with the knee thus being flexed at a right angle. Surface electrodes were used over the soleus muscle. The signals were passed to an optically coupled preamplifier and the results with eight trials were averaged, the closure of the switch in the tendon hammer triggering the averager.

Student's *t* test was used for the comparison of mean values in the various investigations.

RESULTS

Students

Force of contraction. The peak torque was $6{\cdot}3 \pm 2{\cdot}6$ N m for the men ($n = 52$) and $6{\cdot}3 \pm 2{\cdot}6$ N m also for the women ($n = 45$).

Seventeen men were tested with the reduced dorsiflexing torque; their mean peak torque was $4{\cdot}7 \pm 2{\cdot}2$ N m. Fourteen women tested under the same conditions gave a value of $4{\cdot}4 \pm 1{\cdot}3$ N m. These values are lower than those with the greater dorsiflexing torque: for the men the difference is significant at $< 0{\cdot}05$, for the women the difference is significant at $< 0{\cdot}02$. The results are in accord with the long-established finding that, within limits, the greater the initial length of a muscle the greater the force. Sale, Quinlan, Marsh, McComas & Belanger (1982) measured the forces produced by electrical stimulation of the calf

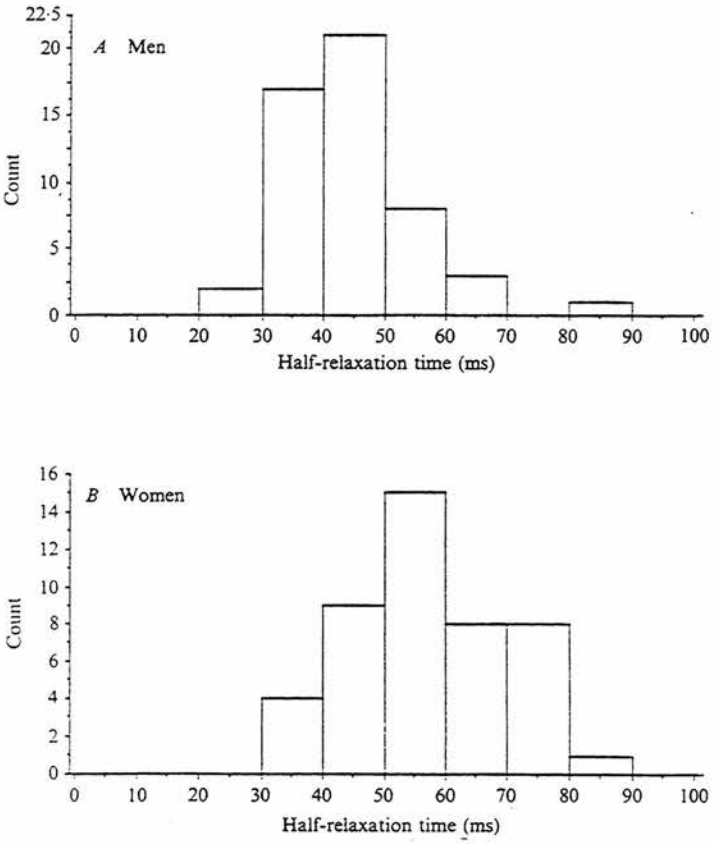

Fig. 3. Histograms illustrating the half-relaxation times in male (*A*) and female (*B*) medical students. There is a clear difference. Statistically the times are longer in the women.

musculature. With the knee bent maximal force development was found with almost full initial dorsiflexion.

The force generated by the contraction at the two levels of dorsiflexing torque in the different students was highly correlated: $r = 0{\cdot}86$, $n = 31$, $P < 0{\cdot}001$.

Half-contraction time. The mean half-contraction time was $27{\cdot}9 \pm 5{\cdot}7$ ms for the men ($n = 52$) and $28{\cdot}9 \pm 4{\cdot}8$ ms for the women ($n = 45$). This difference was not significant.

At the lower dorsiflexing torque the times were $25{\cdot}7 \pm 4{\cdot}7$ ms for the men ($n = 17$) and $30{\cdot}0 \pm 2{\cdot}2$ ms for the women ($n = 14$). There was no significance in the difference.

When the half-contraction times at the two dorsiflexing torques were considered in the different subjects they were found to be highly correlated: $r = 0{\cdot}59$, $n = 31$, $P = 0{\cdot}001$.

Half-relaxation time. The half-relaxation times were $43{\cdot}8 \pm 10{\cdot}7$ ms ($n = 52$) for the men and $56{\cdot}8 \pm 12{\cdot}2$ ms ($n = 45$) for the women. This difference, of the order of 30 %, was highly significant ($P = 0{\cdot}0001$). The data are plotted in Fig. 3.

At the lower level of dorsiflexing torque the values were $39{\cdot}8 \pm 11{\cdot}0$ ms ($n = 11$) for the men and $54{\cdot}3 \pm 13{\cdot}0$ ms ($n = 8$) for the women. This difference too was significant ($P < 0{\cdot}01$).

The data at the two levels of dorsiflexing torque were highly correlated: $r = 0{\cdot}86$,

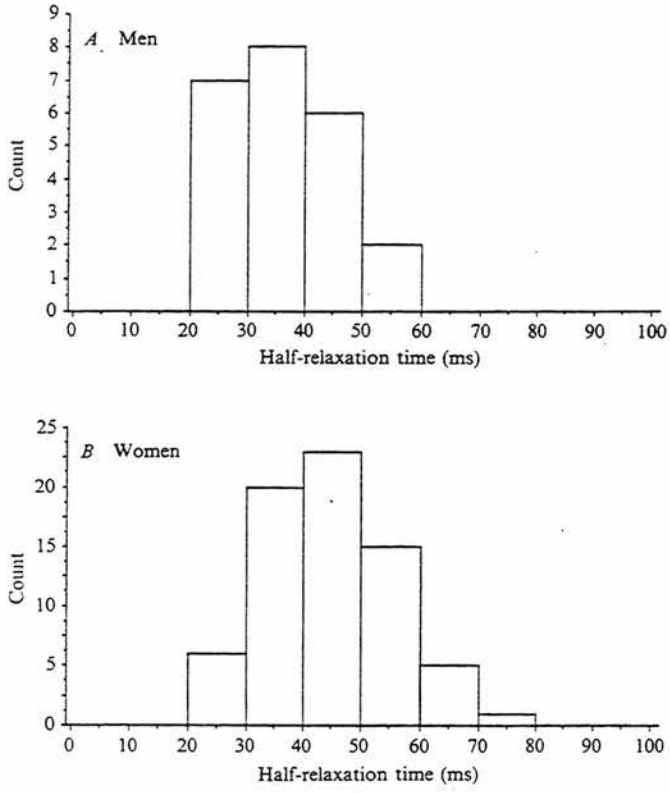

Fig. 4. Histograms illustrating the distribution of the half-relaxation times in the elderly men (*A*) and women (*B*). The difference between the sexes is again of clear statistical significance. The times are longer in the women.

$n = 19$, $P < 0{\cdot}001$. There was, however, no significant correlation of half-relaxation time with the half-contraction time.

Latency and duration of the electromyographic discharge. The latency measured as the interval between the tap and the discharge was found to be $31{\cdot}0 \pm 2{\cdot}8$ ms in the men ($n = 20$) and $28{\cdot}6 \pm 2{\cdot}2$ ms in the women ($n = 15$). This difference, while small, is clearly significant ($P = 0{\cdot}005$). The duration of the discharge was $16{\cdot}0 \pm 4{\cdot}5$ ms in the men and $16{\cdot}8 \pm 4{\cdot}5$ ms in the women. There is no significant difference between these figures.

Elderly subjects

Force of contraction. The 4 A current used for the students usually did not provide forceful enough dorsiflexion to allow for adequately strong reflexes to be obtained in the elderly subjects. Accordingly the dorsiflexing torque was varied to obtain maximal contractions, and the values quoted below relate to the means of four determinations under those circumstances.

There was no significant difference between the optimum dorsiflexing torque for the sexes. The values were, for males, $8{\cdot}7 \pm 2{\cdot}1$ A ($n = 23$), and for females, $8{\cdot}2 \pm 2{\cdot}0$ A ($n = 70$). The peak torques generated were, for males, $3{\cdot}6 \pm 1{\cdot}4$ N m ($n = 23$), and for females, $4{\cdot}3 \pm 1{\cdot}7$ N m ($n = 70$). This 17% difference was of statistical significance ($P = 0{\cdot}03$).

Half-contraction times. The half-contraction times were essentially the same for the men and the women the figures were $25{\cdot}2 \pm 8{\cdot}1$ ms ($n = 23$) for males and $25{\cdot}4 \pm 7{\cdot}7$ ms ($n = 70$) for females. The values are very similar to that found in the younger population.

Half-relaxation times. The half-relaxation times were $36{\cdot}4 \pm 8{\cdot}6$ ms ($n = 23$) for the males and $44{\cdot}3 \pm 11{\cdot}1$ ms ($n = 70$) for the females. This 22% difference is clearly of statistical significance ($P = 0{\cdot}001$) and is illustrated in Fig. 4. There was no correlation with the values for the half-contraction times.

Anthropometry. When the data for men and women were considered separately neither the height, weight, leg length or calf circumference showed any significant correlation with the peak torque, half-contraction time or half-relaxation time.

To estimate the torque, not in absolute terms as above but in relation to body size, the values were divided by the leg lengths. The mean values were, for males, $4{\cdot}5 \pm 1{\cdot}9$ N m m^{-1} ($n = 21$) and, for females, $6{\cdot}0 \pm 2{\cdot}4$ N m m^{-1} ($n = 66$). The difference is highly significant ($P = 0{\cdot}005$). It is apparent that in relation to body size the women produced clearly stronger contractions.

DISCUSSION

An early investigation of the ankle reflex in myxoedema was that of Chaney (1924) who used a pneumatic recorder and a kymograph. Of the clinical investigations since that time many are flawed in the instrumentation. Most have been isotonic, one popular form has used a photoelectric recorder (Gilson, 1959). The swing will have been influenced by the weight of the foot, the position of the centre of gravity, the inertia about the ankle and the passive elasticity of the dorsiflexors. All of these are extraneous factors. Further confusion has been introduced when the investigator has recorded the voltage generated in a coil of wire by the movements of a magnet attached to the foot (Lawson, 1958; Nuttall & Doe, 1964; Abraham *et al.* 1966). This signal will correspond to velocity, but not at all accurately as the distance of the magnet from the coil will vary. 'Confusion is worse confounded' with yet further ambiguity as, usually, the time measured has been the interval between the tap itself and the point at which relaxation is half-achieved. In this way is introduced the time taken up in the reflex pathways and the contraction time; some authors have called such values the 'half-relaxation time'. This is misleading.

Isometric recordings were used by Lambert *et al.* (1951) and are far better. These authors depended on measuring the records of contraction but again, however, their measurements were made from the time of the tap.

We believe that the present system has clear advantages, not least that it is possible rapidly to obtain considerable numbers of determinations to aid statistical analysis, and also that the timings are restricted to relevant parts of the muscular activity.

An increase in the percentage of slow twitch fibres of the vastus lateralis muscle with age has been found by the biopsy of healthy male volunteers (Larsson, Sjödin & Karlsson, 1978), and, using electrical stimulation, Vandervoort & McComas (1986) found a prolongation of the contraction and half-relaxation times of the ankle plantar flexors with age. However, when the bellies of different components were tested individually it was found that, in older people whilst the times for the lateral and medial gastrocnemii were significantly longer, ageing had apparently no effect on the soleus.

In the position adopted in these observations the gastrocnemius will have been relaxed as the knee was bent and it is believed that the results represent activity in the soleus. Levy (1963) studying the EMG concluded that the main activity in the ankle jerk was due to the soleus. The soleus muscle is composed predominantly of slow twitch fibres. According to

Gollnick, Sjödin, Karlsson, Jansson & Saltin (1974) these make up 64–100% of the fibre population. Similar results were reported by Fugl-Meyer, Sjöström & Wählby (1979), who also summarized the findings of other workers.

In the present data there was no evidence of slowing in the older people; many of the students may have been largely sedentary whilst this group of elderly people were quite active physically, being on a fitness week run by 'Saga' holidays.

Women have only about 60% of the muscular strength of men as measured by a hand grip method. The difference between the sexes is found in childhood and increases during adolescence (Newman, Pearn, Barnes, Young, Kehoe & Newman, 1984). It appeared surprising therefore that there was no difference in the peak torques between the young men and women. Furthermore the elderly women produced peak torques which were somewhat higher than those of the elderly men and very clearly greater when considered in relation to leg length.

Women are not only less muscular but also are on average somewhat smaller than men. It is, however, unlikely that either muscularity or size accounts for the sex difference of the relaxation times because when the data for the men and women were considered separately there was no relationship between the anthropometric measurements and these figures. The wearing of high heels by the women was quite exceptional in both groups.

Histographic analyses of the biceps and vastus lateralis muscles were undertaken by Brooke & Engel (1969*a*). The fibre diameters were smaller in the women. Muscle biopsies of the gastrocnemius muscle were undertaken by Costill, Daniels, Evans, Fink, Krahenbuhl & Saltin (1976). They studied samples for seventeen female and twenty-three male track athletes. The cross-sectional area of both the slow and fast fibres was significantly less in the women. Similar findings were noted when comparing the results of biopsies of eleven untrained men and ten untrained women. Examinations of the trapezius muscle, too, have shown that both slow and fast fibres are smaller in women (Lindman, Eriksson & Thornell, 1991). The differences between the sexes are established after the first decade of life (Brooke & Engel, 1969*b*) and then are persistent. Thus in octogenarians both the fast and slow twitch fibres of biceps brachii and vastus lateralis are significantly smaller in women (Grimby, Danneskiold-Samsø, Hyvid & Saltin, 1982).

With the EMG data from the students a significant difference in latency was noted. The longer latencies in the men are no doubt related to the higher average height of men, the conduction distances to and from the spinal cord being somewhat longer. The difference was just under 10%. That the events in muscle start a little later in men should in no way influence the time course of the contractions once they are initiated.

The duration of the EMG discharges are comparable with that of the electrical activity in a single motor unit. Any dispersion of the discharge is thus obviously trivial. In no way can the differences in the relaxation time be ascribed to differences in dispersion of the discharge between the sexes for there was no significant difference in the duration of the discharges. The results accordingly substantiate the view of the numerous earlier investigators who have regarded measurements following a tendon tap as a reliable method of obtaining data about muscle properties.

The very clear difference in the relaxation times between the men and the women of both age groups has implications for the interpretation of data from clinical studies. In their observations on the twitches generated in the plantar flexors by electrical stimulation Vandervoort & McComas (1986) noted that at all ages tested, ranging from young adults to those who were quite old, the half-relaxation times were significantly longer in the women. The present observations extend and amplify these findings.

Whilst it appears that the slower relaxation times in the women are associated with a smaller fibre diameter, an explanation of the cause of the difference in molecular terms is outside the scope of this investigation. Presumably the reuptake of calcium by the sarcoplasmic reticulum is more rapid in men.

The observations were a preliminary background for similar work on children with cerebral palsy, designed to throw light on possible changes in muscle function as a result of that condition (see Wall *et al.* 1964). The work with students was facilitated by Dr N. MacLeod and the authors wish to thank the subjects for their enthusiastic co-operation in this study. As the procedure was trivial and carried no foreseeable risk, ethical permission was not sought. The cost of components for the instrument was defrayed by funds from the James and Grace Anderson Trust. E.G.W. and A.D. acknowledge with thanks support from the Dale Fund of the Physiological Society for the studies of the older people. Figure 1 was drawn by Lesley Skeates-Bailey, medical artist, Department of Child Life and Health, Edinburgh University and was based on a photograph by Mr. L. Cumming.

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Davies,A.(1992)

Localization of the sensation of impact.

Consultancy report for British-American Tobacco Co.

Localization of the Sensation of Impact

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Summary

The sensation of impact of inhaled tobacco smoke , as defined by Dr.P.C.Bevan (B.A.T. Position Paper of June 1990), was anatomically localized in the upper respiratory tract of seven subjects. Spirometry was used to define the volume of inhaled smoke required to produce impact. Subsequent local anaesthesia of the pharynx and soft palate excluded these structures from the sensation while the volume was again determined. The validity of spirometric techniques to determine the likely anatomical site of impact was supported by morphometric and physiological considerations, and the observation that although cigarettes of different impact ratings produced different subjective responses the volumes of smoke to produce impact were not significantly different.

The results suggest the sensation of impact arises from airways distal to the larynx.

From patterns of breathing produced by smoke inhalation it is likely that this sensation arises from rapidly adapting receptors.

Introduction

Impact of tobacco smoke has been defined as " the transient hit or kick felt in the throat when tobacco smoke is inhaled"(Bevan,1990).

As with other subjective sensations it is probable that it varies from subject to subject. It was unnecessary to attempt a more precise definition for this study since our subjects, all smokers, claimed to be familiar with the sensation even before coaching with cigarettes of different impacts.

The primary objective of this study was to identify the anatomical region of the tracheobronchial tree from which impact arises.

This was achieved by measuring the volume of smoke that needed to be inhaled to just trigger the sensation. Seven subjects gave their informed consent to the study; their smoking history and physical characteristics were defined. Lung parameters were measured as will be described in Methods and the volume of smoke inhaled to provoke impact measured. A subsidiary observation was that the subjects then inhaled a further volume of air, greater than their normal tidal volume as part of the smoking breath, this volume was also measured.

Smokers report that the sensation of impact arises from "the back of the throat", the sensation seems to arise from, the pharynx. This is reasonable since this region is rich in superficial sensory receptors of the type which would fulfil the criteria for generators of sensations like impact. To see if the pharynx and soft palate were necessary for impact these structures were anaesthetized with lignocaine and the volume of smoke required to provoke impact, if the sensation persisted, measured again.

Methods

Relevant physical characteristics and smoking history of the 7 subjects involved in this study were recorded. Table 1.

Their Vital Capacities (VC) were measured on a Benedict spirometer, clinical practice of taking the largest of 3 test results was followed.
The subjects were then allowed to sit at the spirometer for 5 minutes to acclimatize themselves to the sensation before their rest tidal volume was measured as the average of 5 breaths.

The subjects were then taken through a training session where the sensation of impact was discussed and demonstrated with the aid of BAT cigarettes of 1 and 5 impact rating. The experimental procedure to be followed was explained, and when the subjects were completely informed they were taken to the experiment room.

The experiment consisted of the subject breathing out to resting Functional Residual Capacity (FRC). The subject then inhaled from an impact 1 cigarette taking smoke and air ad libitum to the point of impact. He then exhaled thought a Fleisch Pneumotachograph-head back to FRC. The pneumotachograph head was connected to an electronic integrator which calculated the volume exhaled (Volume A). The procedure was repeated 5 times by each subject.
It was noted in course of preliminary experiments that the subject, if not required to immediately exhale through the pneumotachograph, would draw in more, fresh air before exhaling. We tested the suggestion that this breath, which resembles normal smoking practice was significantly larger than a normal resting tidal volume. This was done by allowing the subject to inhale a complete breath, (impact volume plus the additional volume) and then exhale back to FRC through the pneumotachograph (Volume B). The procedure was repeated 5 times.

To test if impact strength of the cigarette influenced the inhaled volume required to produce impact both parts of the experiment were repeated with a Strength 5 cigarette.

After the experiment had been repeated with the Strength 5 cigarette 5 subjects agreed to have their soft palate topically anaesthetized. In one of these subjects the gag reflex produced by her anaesthetized soft-palate was too strong for her to continue with the experiment.

Topical anaesthesia was produced by a spray of 10% Lignocaine (Xylestesin, ESPE Pharmaceuticals) applied to all the soft-palate and oropharynx visible under direct vision with a laryngeoscope. Anaesthesia was considered complete when the subject could not detect light probing of the pharynx wall with a blunt glass rod.
After anaesthesia the impact experiment was immediately repeated with the Strength 5 cigarette

Differences in results were considered significant when $p<0.05$ using two-tailed t-test.

RESULTS

Table 1

Physical Characteristics of Subjects

Subject	Age	Height(cm)	Wt.(kg)	Years Smoked	No/day Smoked	Total Smoked	Tar
1.(F)	33	165	62.7	18	15	98550	H
2.(F)	20	164	57.2	7	20	51100	L
3.(F)	19	161	61.4	4	10	14600	L
4.(F)	52	160	60.0	20	13	94900	L
5.(F)	19	165	56.0	7	10	25550	L
6.(M)	27	172	63.6	14	30	153300	H
7.(M)	47	180	76.4	26	18	170820	L

Table 2

Impact Volumes
Pre-Anaesthesia

Vol. A = Impact volume (ml.)
Vol. B = Total Breath volume (ml.)

Subject	VC	V_T	Impact 1		Impact 5	
			Vol.A	Vol.B	Vol.A	Vol.B
1.	3000	543	270	900	245	919
2.	2256	916	245	1489	127	744
3.	3953	721	306	952	254	714
4.	1546	634	204	613	194	430
5.	2591	589	188	960	225	903
6.	2485	371	285	1097	306	1081
7.	4274	716	230	699	204	698

During Anaesthesia

Subject	Impact 5	
	Vol.A	Vol.B
1.	250	806
2.	163	711
3.	333	825
5.	258	1058

All volumes, in ml, are the mean of 5 tests. For clarity standard errors are not given for individual subjects but are given in Table 3 where Grand Means are calculated using the 5 tests provided by each subject before and during anaesthesia.

Grand Means ±S.E.

Vital Capacity	Tidal Volume	Impact 1		Impact 5	
		Vol.A	Vol.B	Vol.A	Vol.B
872 ±362	642 ±64	247 ±16	958 ±108	222 ±21	784 ±79

During Anaesthesia

251 ±35	850 ±73

These results show:

1. The volume of smoke to produce impact = 247 ± 16ml.
2. The total volume of a smoking breath (958 ± 108ml) is greater than that of a normal breath (642 ± 64ml).
3. Impact volumes and breath volumes are not statistically significantly different for Impact 1 or 5 cigarettes.
4. Local anaesthesia of the soft palate and pharynx does not affect impact or smoking breath volumes.

DISCUSSION

Methodology

Despite an enormous literature on the subject surprisingly little is certain about smoking patterns, mainly because of the absence of a reliable non-obtrusive monitoring technique to measure puff volume and subsequent inhalation pattern. Specialized cigarette holders (Guillerm and Radziszewski, 1978), head-out volume-displacement plethysmography (Adams, Lee, Rawbone, and Guz, 1981) and inductive plethysmography (Tobin and Sackner, 1982) are but a few of the techniques used by previous workers. The techniques of course were determined by what particular volumes and patterns were being measured. Measurement of puff-volume, for example, because of the small volume involved requires orders of magnitude of accuracy greater than measurement of vital capacity sized volumes to produce meaningful results.

It seems that the requirement that smoking behaviour patterns should not be interfered with is as important as the accuracy of the monitoring devices that were used (Sackner,M.A. 1980).

In the present study because of the morphometry of the region in which impact was triggered a high degree of volume resolution was found to be unnecessary (vid inf).The problem of interfering with the behaviour pattern does not arise because the smoking breath is taken freely before any instrumentation is involved.

Morphometry

Any attempt to localize of the sensation of impact within the airways must be based on a sound knowledge of the morphometry of the human trachiobronchial tree. Two models were used as a source of morphometric information in this study, that of Weibel (1963) and Horsfield (1981). Both models agree in terms of volumes and anatomical structures of the volumes we are interested in. Of particular note, in terms of the effect of lung structure on inhaled gases and vapours, is the increase in cross-sectional area peripherally and, furthermore, that the rate of increase of cross-sectional area is greater peripherally. Thus the mean velocity of flow of gas in the conducting airways rapidly falls towards the respiratory zone (Davies, 1975), but at the same time molecular diffusion becomes increasingly effective with increasing cross section over which to operate. This effect of increasing cross-section has implications for diffusion of tobacco smoke and, more importantly for this study, for the accuracy of anatomical localization of inhaled volumes. Thus at inhaled volumes of the order of 100mls an error of say 2.5ml will result in a an error of the estimated position of the gas front of 1cm. in the trachea. At inhaled volumes of the order of 200ml errors of 2.5ml will result in positional errors of 0.1cm in the terminal bronchioles. Thus at the volumes impact was detected in this study the linear position of the smoke front in the bronchial tree, and hence the nature of the airways in which it was situated, was being estimated with a high degree of accuracy. The robust nature of the measurement of volume in our estimated of position also minimises the effect of the possibility of subjects not breathing out to the

same FRC from which they began the inhalation of tobacco smoke. The expiratory reserve volumes of our subjects could be estimated to be of the order of 700ml (Cotes, J.E. 1974). That would be the maximum volume error they could consciously contribute to an overestimate of the position of impact. It is more likely that they might make errors of 10% of this, which would result in maximum errors of less than 3cm in the localization of the position of receptors responsible for the sensation of impact.

It may be suggested that the nature of the interface between the inhaled and deadspace air in the lungs might blur estimates of position. Laminar flow in a tube results in a central core of advancing gas while a shell of residual gas lines the walls. This would obviously make estimates of position of a concentration of smoke that would trigger impact difficult. It is unlikely that lamina flow exists to any extent in the upper airways .This would seem to contradict the universal finding that patients can be adequately ventilated by volumes less than their morphometrically calculated dead space. An important factor enabling this to take place is the churning action of the heart mixing all gas below the carina. Thus in many patients alveolar gas can be found at the carina at the beginning of expiration (Nun and Hill, 1960). Anatomical dead space is thus the <u>functional</u> inhaled volume to reach the end of the conducting airways. If we assume a bolus of smoke behaves in the same way as inhaled air, and this must be done with caution because of the separating effect of the bronchial tree on gas mixtures (Horsfield, Davies and Cumming, 1979) we can apply the clinical rule of thumb "a patients anatomical dead space is his weight in pounds" (Radford,1955) and take 150 ml. as the functional volume to reach the end of the conducting airways.

<u>Receptors</u>

The location of somatic sensation is notoriously unreliable. This is most dramatically seen in clinical situations where visceral pain is often felt not in its 'true' position but <u>referred</u> to the region of the body that shares the same dorsal root: thus pain is felt in the groin in response to a stone in the ureter, and in the left arm in angina pectoris. The subjective report of impact arising from "the back of the throat" should therefore be treated with caution. It is just conceivable that the sensory endings of the nose may be involved in the sensation of impact due to convective mixing of inhaled smoke with air in the nasopharynx. This seems extremely unlikely and it suffices to say that receptors in this region which respond to olfactory sensations are described by Berglund and Lindval (1982) while other sensory nerves, which seem to be largely neuropeptide in nature are described by Uddman and Sundler (1986).

These nerves respond to a wide spectrum of stimuli including cigarette smoke which causes discharge in the trigeminal nerves (Ulrich et al.,1972). It is unlikely however because of the pattern of inhalation in smoking, and the volumes involved in triggering impact in the present study, that these receptors are involved in the sensation.

Subjective reports of the nature of impact would suggest the pharynx as its origin. Although much is known about reflexes from the pharynx little is known about the appearance of nerve endings there or their pattern of discharge. Only one type of epithelial receptor has been described morphologically (Fillenz and Widdicombe, 1971) although it must be remembered that the muscles of the pharynx will contain muscle spindles characteristic of skeletal muscle. The epithelial receptors described by Fillenz will have certainly been anaesthetized by the lignocaine used in the present study and the sensation of "a foreign body in the throat" reported by our subjects after local anaesthesia suggests deeper receptors were blocked as well. Study of pharangeal receptors has concentrated on their response to mechanical deformation (Hwang et al.,1984) although there is some evidence of chemical sensitivity (Nail et al.,1969). Resistance to local anaesthesia and the volumes of smoke required to trigger impact in the present study militate against these receptors being the origin of impact.

It is an universal experience that the larynx is a potent source of sensations and reflexes. Of the three distinct routes followed by laryngeal afferents (internal branch of superior laryngeal nerve, external branch of the same nerve and the recurrent laryngeal nerve) the internal branch of the superior laryngeal nerve is by far the most important. This carries afferent information from articular (joint) receptors, muscular receptors and the glomerular corpuscular nerve endings and epidermal free nerve endings (Ardouin and Maillet,1965) which are the types that most warrant our attention in relation to the present study.

Exposure of the laryngeal mucosa to tobacco smoke produces a slowing of respiration in anaesthetized animals (Lee and Morton, 1987). Interestingly coughing, a typical response to chemical or mechanical stimulation, was not seen by these workers in response to tobacco smoke. In conscious dogs dilute cigarette smoke (50-70%) insuflated retrogradely through the larynx consistently provoked an aspiration reflex- rapid brief inspirations not followed by expirations. This reflex has some features in common with the augmented breath (vid inf) but caution should be exercised in attributing it to laryngeal receptors since it is most usually elicited from nasopharyngeal receptors (Widdicombe,1986) which may have been inadvertently exposed to smoke in that particular experiment due to the retrograde insuflation.

In view of the indicated location of receptors responsible for impact in the present study it is unfortunate that the effects of cigarette smoke on the trachea are not well defined. However one of the most pertinent observations recorded in the literature is that when cigarette smoke was inhaled into the lungs of conscious dogs, via a high cervical tracheostomy, a characteristic response was an immediate augmented breath (a sigh) characterized by a two-step inspiratory flow and a large tidal volume (Lee et al.,1986).

Cigarette smoke has been demonstrated to stimulate rapidly adapting receptors in the airways (Sampson and Vidruk, 1975) and rapidly adapting receptors have been shown to provoke augmented breaths (Davies and Roumy, 1982). The abundant existence of rapidly adapting receptors in the trachea (Sant'Ambrogio and Sant'Ambrogio, 1980) supports the suggestion that they contribute to the large breaths reported to be taken by smokers in response to impact in this and other (Rodenstein and Stanescu, 1985) studies.

Stimuli

It was not the primary objective of the present study to investigate the nature of the stimulus which produces impact. Suffice it to say that nicotine, among the 4,000 or more chemical compounds identified in tobacco smoke (Dube and Green, 1982) is a strong contender. Although nicotine has been demonstrated for some time to be a potent stimulant of peripheral and central chemoreceptors (Heymans et al 1931, McQueen,1983) and receptors in the lungs (Taylor et al, 1986) direct evidence for stimulation of receptors in the region of the bronchial tree identified in the present study is lacking. In view of the suggestion that rapidly adapting receptors in the airways may be implicated in the sensation of impact the observation of Sellick and Widdicombe (1971) that inert particles of micron size could stimulate rapidly adapting receptors should not be neglected.

It is known that smokers adjust the size of puff to the tar content of the cigarette. Tobin and Sackner (1982) noted a difference of 13ml. (52ml.-39ml.) between low and high tar puffs. The identity of volumes to trigger impact in the present study suggests an all or none effect, typical of many respiratory reflexes, which in turn suggests impact may be the sensory expression of the pharmacological triggering of a physiological reflex.

Other studies

Although cigarette smoking pattern has been investigated by a number of workers (e.g. Tobin et al,1982; Adams et al 1983; Rodenstein and Stanescu, 1985), the location of the origin of the sensation of impact has not apparently been previously addressed.

To measure the volumes they were interested in specialized cigarette holders (Guillerm and Radziszewski, 1978), head-out volume-displacement plethysmography (Adams, Lee, Rawbone and Guz, 1981) and inductive plethysmography (Tobin and Sackner,1982) are but a few of the techniques used by previous workers. Most of the results obtained divided smoking pattern into 2 phases, the puff and the subsequent breath. In fact 3 phases need to be recognised. I would suggest that smokers not only identify the moment of impact but they alter their pattern of inhalation at that time depending on the nature of the sensation they have received. Thus the three phases that need to be identified are-

a) Puff volume b)Impact volume c) Full inhalation volume

Tobin et al (1982) correctly identified the fact that inhalation of cigarette smoke altered the nature of the subsequent breath. They unfortunately related the volume of these breaths to their subjects vital capacities, perhaps on the basis that vital capacity is one of the most repeatable respiratory measurements, in what they called "the inhalation fraction". If they had used the less repeatable, but more physiologically significant, tidal volume of their subjects at rest they might have observed that their subjects were taking a significantly greater number of augmented breaths after each puff as illustrated but not identified as such in their Fig.1 p.698.

Rodenstein and Stanescu (1985) used qualitative measurements of nasal and oral air-flow to characterize pattern of smoking. Their Fig.2, p.629 shows double inspiratory nasal flows, not separated by an expiration, associated with a large tidal volume. These are the characteristics of augmented breaths seen in animals (Davies and Roumy, 1982) although the time course of events appears different. This difference may be explained by the time constants of the recording devices use in Tobins experiments. The evidence of these workers, along with our own, suggests that inhalation of tobacco smoke reflexly produces augmented breaths in human smokers.

CONCLUSIONS

Measurement of the volume of inhaled smoke required to produce the sensation of Impact suggests that its origin is distal to the pharynx and is probably situated in, or distal to, the trachea.

Local anaesthesia of the soft-palate and oropharynx excluded these structures from the sensation.

Established lung morphometry suggests that in the region originating Impact smoke moves as a bolus producing a rapid increase in smoke density as the smoke/residual air interface passes.

Breathing patterns recorded in this and other studies suggest Impact is the sensory expression of the triggering of an all-or-none physiological reflex.

The most likely sensory receptors transducing the sensation of Impact are the epithelial rapidly adapting receptors situated in the conducting airways. This conclusion is supported by anatomical, pharmacological and physiological evidence.

RECOMMENDATION

The different effects of high and low Impact cigarettes on pulmonary rapidly adapting receptors should be investigated with the objective of establishing a rational policy for maximising impact while minimising irritation and harmful constituents of cigarettes.

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An inexpensive ultrasonic aerosol generator.

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An inexpensive ultrasonic aerosol generator

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Inhaled aerosols are used as experimental and therapeutic tools (Lourenco & Cotromanes.1982). Ultrasonic aerosol generators offer many advantages over jet types. They produce a dense cloud, and therefore can deliver a high dose in a short time. The distribution of droplet size they produce, and therefore the sie of deposition in the lungs, is narrower (Sterk *et al.*1984). They do not require a supply of compressed air (Wright.1958) and do not pressurize systems to which they are attached.
Their major disadvantage is that they are relatively expensive compared to other types and usually require a relatively large charge of liquid to be aerosolized.
I will demonstrate an inexpensive ultrasonic aerosol generator, constructed from a commercial humidifier, which requires only small amounts of liquid to function.

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The ankle jerk in young men and women.

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The ankle jerk in young men and women

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In earlier work we measured the mechanical events following a tap to the Achilles tendon in students. For both men and women we used throughout the observations steady dorsiflexing torque of 1·12 N m to induce some tension in the soleus muscle and thus allow the tap to be effective (Walsh *et al.* 1993). We found that the relaxation in the women was significantly slower than that of the men.

It is known, however, that the relaxation time of muscle varies with its length, becoming longer when the tissue is elongated (Hill, 1972; Sale *et al.* 1982). As women are on average less muscular than men our results might have been due to the dorsiflexing force inducing a greater degree of stretch of the muscle in the women.

In the present investigations we have used the same apparatus as was described previously but for each subject tested we adjusted the dorsiflexing force to bring the foot to rest at a right angle to the leg. We have thus controlled the length of the muscle. Our observations were on 85 male and 91 female first year students during their practical work in physiology. The half-relaxation times (mean ± S.D.) were 35·7 ± 7·9 ms in the men and 41·2 ± 9·2 ms in the women. This difference is illustrated in Fig. 1 and is highly significant ($P = 0·001$). The mean age of both groups was 18·5 years.

Thus under these different conditions a similar difference between men and women has been found, as has previously been reported.

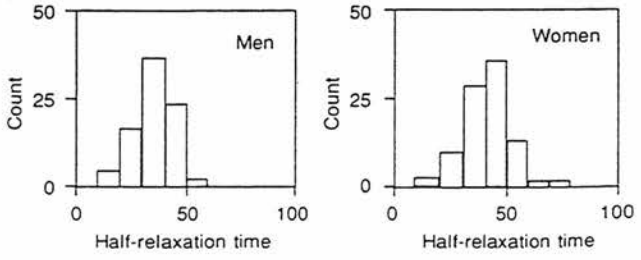

Fig. 1. Frequency distributions of the results. The abscissae refer to the times taken for the tension to fall from 75 to 25 % of the peak value.

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A novel ultrasonic aerosol generator.

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A novel ultrasonic aerosol generator

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ABSTRACT

An ultrasonic aerosol generator constructed from a domestic humidifier is described which has been used to produce liquid aerosols for physiological investigations. The instrument was constructed from a Pifco™ domestic humidifier modified to include an energy guide to direct the oscillations of the transducer through the coupling water, which would normally be aerosolized, onto a small membrane based sample chamber containing the liquid to be aerosolized. The size distribution of the aerosol produced was found to be between 2 and 6 mm, optimum for diffuse intrapulmonary deposition. Up to 4 ml/min of aqueous liquid was used; however the sample chamber could be made small enough to contain economic amounts of expensive material to administer by inhalation. The instrument has proved to be reliable over a period of three years.

Keywords: Aerosol, aerosol generator, inhalation therapy

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INTRODUCTION

Inhaled aerosols are extensively used in medicine, veterinary medicine and respiratory physiology as experimental and therapeutic tools. The inhalation of aerosols of aqueous solutions plays an important role in both the diagnosis and treatment of a variety of respiratory disorders. In the aetiological diagnosis of asthma for example inhalation provocation tests are essential in determining the degree of immunological or non-specific airway reactivity[1,2]. In these tests, allergen extracts, some of which can only be prepared with difficulty, are aerosolized. It is therefore useful if as small a volume of solution as possible can be used.

In the treatment of bronchial asthma, bronchitis, bronchiectasis, cystic fibrosis and bronchopneumonia, inhaled aerosols are used with varying degrees of success[3,4]. Important determinants of efficacy of drug delivery in such studies include particle size distribution and density of the aerosol. Drugs administered by inhalation include, among others, bronchodilators, mucolytics, antimicrobials, steroids and disodium cromoglycate. The biological characteristics of the respiratory system of the patient or subject under investigation interact with the characteristics of the inhaled aerosol which in turn depend highly on the characteristics of the aerosol generator used.

The two major types of generator used at present are the jet, driven by compressed air, and the ultrasonic type which relies on a driven element vibrating at ultrasonic frequency in the liquid to be aerosolized. Whatever the method of production the major characteristics of an aerosol of interest to a respiratory physiologist or clinician are the distribution of sizes of its droplets (determined largely by surface forces) and the density of the cloud, that is the amount of liquid suspended in a given volume of air.

Ultrasonic generators offer advantages over jet types in terms of these and other characteristics. They produce dense clouds, and therefore can deliver a high dose in a short time. The distribution of droplet size they produce, and therefore the site of deposition in the lungs, is narrower[5]. They do not require a supply of compressed air[6] and do not pressurise the system to which they are attached. Their major disadvantages are that they are expensive compared to other types and usually require a relatively large charge of liquid to be aerosolized.

To overcome these problems we have modified a Pifco™ Domestic Humidifer (Pifco Ltd., Sailsworth, Manchester, M35 0HS) to aerosolize small (down to 5 ml) samples of liquid. We have used this instrument for more than three years in studies requiring deposition of particles in the lungs.

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Table 1 Raw data for Figure 2

Size microns	% under	% in band	Size microns	% under	% in band
188	100	0.00	17.7	100	0.00
162	100	0.00	15.3	100	0.00
140	100	0.00	13.2	100	0.00
121	100	0.00	11.4	100	0.00
104	100	0.00	9.82	100	0.00
89.8	100	0.00	8.47	100	0.00
77.5	100	0.00	7.30	100	4.02
66.8	100	0.00	6.30	96.0	28.3
57.7	100	0.00	5.43	67.7	34.0
49.8	100	0.00	4.68	33.8	23.1
43.0	100	0.00	4.05	10.6	10.3
37.0	100	0.00	3.48	0.36	0.36
32.0	100	0.00	3.02	0.00	0.00
27.5	100	0.00	2.60	0.00	0.00
23.8	100	0.00	2.23	0.00	0.00
20.5	100	0.00	1.93	0.00	

Result source = Averaged
Record No. = 36
Focal length = 100 mm
Presentation = lds
Volume distribution
Beam length = 20.0 mm
Obscuration = 0.2557
Volume conc. = 0.0022%

Normal
X = 4.96, N = 0.40
D(v,0.5) = 5.06 μm
D(v,0.9) = 6.02 μm
D(v,0.1) = 4.03 μm
D(4,3) = 5.04 μm
D(3,2) = 4.94 μm
Span = 0.4
Spec. surf. area
1.2143 sq.m./cc.

METHODS

The aerosol generator is shown in section in *Figure 1*. The parts that have been added to the original domestic humidifier are shown in cross-hatched section and are constructed out of perspex. The original humidifier consisted of those parts shown (not cross-hatched) plus a simple plastic box containing a water bottle, which provided a constant level of water over the ultrasonic transducer (9). The box also prevented splashing of large particles out of the system. The box and water bottle were discarded for our instrument.

In its original form the ultrasonic transducer dissipated its energy throughout the surface of the water placed in the humidifier. In the modified instrument the energy is focused by a tubular energy-guide (8) on the base of the sample tube (4). The lower end of the tube consists of a thin rubber diaphragm (7) made of a piece of a child's balloon tied in place with thread and immersed in the coupling-water (2) which covers the transducer.

An air-supply (1), part of the original humidifer, consists of a small fan which provides a controllable stream of low pressure air. This is contained within the air-box (5) and admitted to the sample-tube by two holes at its top. The air stream flows down the sample-tube and up the baffle-tube (3) which prevents the escape of large drops of liquid and returns them to the body of the sample.

The characteristics of the aerosol were determined by passing it, immediately it left the generator, through the beam of a Malvern 2600 laser defraction droplet size analyser (Malvern Instruments, Spring Lane, Malvern, WR1 1AQ). In this the sample is illuminated by a 2 mW, 632.8 nm helium–neon laser. The particles scatter the light at angles which are characteristic of their size, forming a series of diffraction patterns. The scattered light is collected by a Fourier optical system and focused on a detector made up of a series of diode elements. The signal from each detector is amplified, digitized and the complete light energy pattern then analysed by computer.

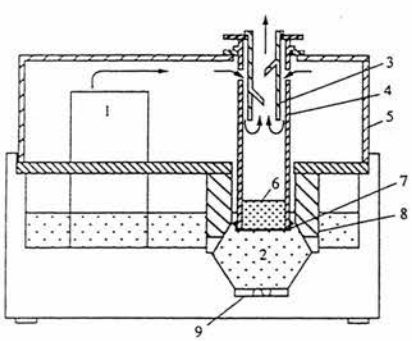

Figure 1 Vertical section of aerosol generator showing: (1) air supply, (2) coupling water, (3) baffle tube, (4) sample tube, (5) air box, (6) sample, (7) diaphragm, (8) energy guide, (9) ultrasonic transducer. The airflow is shown arrowed

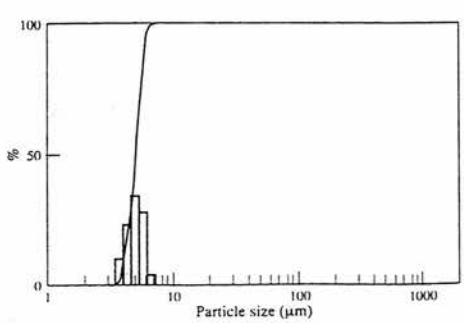

Figure 2 Printout of Malvern 2600 Droplet Size Analyser showing distribution of size of water droplets produced by the aerosol generator, with a mean at about 5 μm

RESULTS

The amount of aerosol produced, in terms of volume of air and droplet density, could be varied by altering the power supplied to the air fan and transducer respectively using the original controls of the humidifier.

The focused energy of the transducer was sufficiently great, at full power setting, to aerosolize 4 ml of solution per minute. The characteristics of a room temperature distilled water aerosol were 90% of droplets below 6 μm in diameter, 50% of droplets below 5 μm, 10% of droplets below 4 μm, as shown in *Figure 2* and *Table 1*.

This size distribution was not affected by the power setting of the transducer and compares favourably with commercial ultrasonic nebulizers in which median droplet diameters were in the 5.2–6.7 μm range[1].

CONCLUSIONS

We have described an ultrasonic nebulizer constructed from a domestic humidifier. Our instrument produces an aerosol with characteristics comparable to those produced by commercial devices[7], but at about a tenth of the capital cost. The instrument has been successfully used for drug administration and investigations in respiratory physiology for three years, and provides an economic alternative to commercial ultrasonic nebulizers.

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Publication 60.

Davies,A. & Pirie,L.(1995)

Pulmonary receptor activity in anaesthetised emphysematous rats.

J.Physiol.487,112.

Pulmonary receptor activity in anaesthetized emphysematous rats

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We have investigated if changes in pulmonary receptor activity accompany the changes in architecture of the lung in a model of emphysema, produced in eighteen rats by a single intratracheal insufflation of 120 mg kg^{-1} papain in 0·25 ml saline administered under Halothane anaesthesia. After 4 weeks the rats were anaesthetized with 6 ml kg^{-1} of 25% urethane. Single vagal fibre recordings were made from pulmonary receptors during eupnoea and sustained inflations and deflations of the rat's lungs by pressures of ± 0·5 kPa. Initially receptors were classified as slowly (PSR) or rapidly adapting (RAR) by their conduction velocities (36·1 ± 2·5 m s^{-1}, $n = 14$ and 14·5 ± 2·3 m s^{-1}, $n = 8$, respectively). However, the types could be easily differentiated by their response to steps of inflation. The ratio of PSRs to RARs was unchanged from controls in groups of rats investigated at 1, 4 or 8 weeks after insufflation. The ratio of RARs to PSRs (1 : 2) was higher than in slower breathing species, rabbit 1 : 4 (Roumy & Leitner, 1980), cat 1 : 10 (Widdicombe, 1954), and may provide effective vagal feedback to the central pattern generator (Bartlett & St John, 1979). Discharge of PSRs was mainly in inspiration: RARs discharged almost exclusively in expiration. PSRs had a significantly ($P < 0·05$, $n = 250$) higher peak frequency of discharge in the emphysematous rats (91·2 ± 2·0 compared with 86·1 ± 2·0 Hz) and RARs had a significantly ($P < 0·001$, $n = 140$) higher peak frequency and number of action potentials per expiration (118·8 ± 5·7 compared with 87·5 ± 6·0 Hz and 15·1 ± 0·5 compared with 13·3 ± 0·5). On inflation of the lungs with 0·5 kPa ($n = 49$) the number of action potentials per second during the first 0·25 s of inflation was 103·3 ± 4·6 and 112·7 ± 4·7, respectively, for the control and emphysematous rats. The adaption index of the emphysematous rats was significantly ($P < 0·01$) lower (49 ± 2·2%) than that of the control rats (60 ± 3·0%), which explains the exaggerated Hering–Breuer inflation reflex found in the emphysematous rats (Pirie & Davies, 1995).

Results are given as means ± S.E.M.; P was calculated by Student's t test.

Supported by the Salvesen Emphysema Research Trust.

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Publication 61.

Walsh,E.G.,Davies,A.S. & Powers,N. (1995)

Automated measurement of the knee jerk in students and rowers.

J.Physiol. 478,73p.

Automated measurements of the knee jerk in students and rowers

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The person sat with the knee at a right angle, the seat height being 70 cm. The foot rested on a platform fixed to a load cell. Knee jerks were elicited by a tendon hammer equipped with a microswitch which triggered the electronics. The peak force, 'rise time' needed for the force to rise from 25 to 75%, and the 'fall time' for it to fall between these limits, were displayed on digital meters. The contractions were also recorded graphically; several example are shown in Fig. 1.

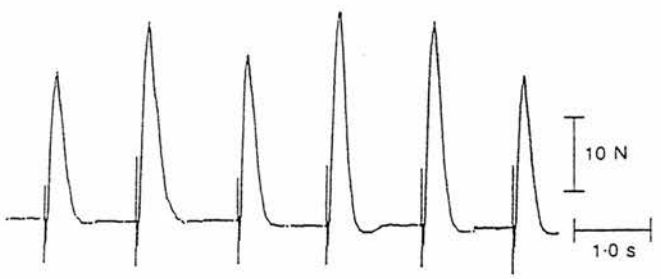

Fig. 1. Examples of successive responses in one person.

When the contraction developed the tension rose abruptly in a quasi-linear manner. The peak of the curve was pointed, there being virtually no plateau. The drop of tension was more gradual and the slope was almost constant until much of the fall had been completed. The results with thirty-eight men and thirty-nine women medical students were compared with ten members of the university boat club (four men and six women). The results (means ± S.D.) were:

	Peak force (N)	Rise time (ms)	Fall time (ms)
Medicals	32·2 ± 8·9	22·1 ± 10·4	55 ± 12·8
Rowers	32·8 ± 8·9	18·2 ± 5·65	60 ± 18·6

The values are close; no differences were significant using the *t* test.

Rowing is an endurance sport, the athletes exercising for several hours weekly on 'ergos' in the gym but the parameters of quadriceps contractions were in no way exceptional.

Publication 62.

Davies,A. & Pirie,L.(1995)

Pattern of breathing and lung reflexes in anaesthetized emphysematous rats.

J.Physiol.487,111P.

Pattern of breathing and lung reflexes in anaesthetized emphysematous rats

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Patients with pulmonary emphysema breathe with a characteristic pattern of increased minute ventilation and breathing frequency (Loveridge *et al.* 1986). Anaesthetized rabbits with emphysema have slower breathing than matched controls (Delpierre *et al.* 1985). We have produced a rat model of pulmonary emphysema (mean linear intercept of the alveolar walls increased from 81 ± 3 to 109 ± 2 μm, $n = 31$, $P < 0{\cdot}01$), by a single intratracheal insufflation of 120 mg kg^{-1} papain in 0·25 ml saline under Halothane anaesthesia. After 4 weeks the eighteen rats were anaesthetized with I.P. urethane (0·6 ml, 25% solution per 100 g body mass). Compliance of the emphysematous lungs at postmortem was $0{\cdot}66 \pm 0{\cdot}08$ compared with $0{\cdot}41 \pm 0{\cdot}04$ ml kPa^{-1} ($n = 9$, $P < 0{\cdot}02$) controls. Arterial P_{O_2} of the emphysematous rats was $12{\cdot}4 \pm 0{\cdot}5$ kPa and of the controls $13{\cdot}4 \pm 0{\cdot}19$ kPa. P_{CO_2} was $6{\cdot}5 \pm 0{\cdot}3$ and $5{\cdot}8 \pm 0{\cdot}3$ kPa, respectively. Eupnoeic pattern of breathing measured over five breaths in the emphysematous rats was not significantly different from the controls.

After bilateral vagotomy inspiratory time (t_I) increased to $0{\cdot}63 \pm 0{\cdot}07$ and $0{\cdot}61 \pm 0{\cdot}01$ s in the emphysematous and control, and expiratory time (t_E) to $1{\cdot}30 \pm 0{\cdot}14$ and $1{\cdot}42 \pm 0{\cdot}2$ s, respectively. These changes in t_E on vagotomy suggest a more powerful t_E extending vagal mechanism exists in the emphysematous rats than in the controls. This is reinforced by results of the Hering–Breuer inflation reflex. An intratracheal pressure of 0·5 kPa produced a Hering–Breuer ratio of $9{\cdot}1 \pm 1{\cdot}8$ ($n = 27$, $P < 0{\cdot}05$) in the control and $27{\cdot}8 \pm 2{\cdot}8$ ($n = 36$) in the emphysematous rats. Lung deflation by −0·5 kPa typically resulted in a sustained increase in duration of t_I and shortening of t_E. However, 11% of control rats responded to lung deflation with a shortening of t_I and t_E. In emphysematous rats, 19% responded in this manner. Our results provide an explanation for the results of Delpierre *et al.* (1985), and the reduced tidal volume seen in emphysematous horses (Gillespie *et al.* 1966) and further evidence of the lack of importance of the Hering–Breurer reflex in humans.

Means and S.E.M. are used throughout. P was calculated by Student's t test.

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Publication 63.

Dallak,M.,Xiujie Luan,Davies,A.,Brown,H. &
Pirie,L.(1995)

Stability of breathing patterns in men rats and rabbits.

J.Physiol.483,96P.

97P
Journal of Physiology (1995)483.P

Stability of breathing patterns in men, rats and rabbits

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We intend to compare breathing pattern in conscious emphysematous and normal rabbits. To estimate the minimum number of emphysematous rabbits required to give a statistically valid result we need to know the variability of the pattern of breathing in normal rabbits.

We can find no record of such a measurement in the literature, although rabbits are said to have a highly labile pattern of breathing. The reproducibility of pulmonary mechanics has been measured in cattle (Galivan & McDonell,1988) and breathing pattern of human beings has been measured in single instances (Tobin *et al.*1983) and over time (Benchetrit *et al.* 1989).

We have adapted the whole body plethismography method of Bartlett & Tenney (1970) developed from the method of Drorbaugh & Fenn (1955) to measure the breathing pattern of four rabbits and four rats on four separate days. The plethysmograph consists of a chamber through which a stream of air enters from a pump and leaves via a narrow tube. This tube offers high impedance to flow at the frequency of breathing of rats and rabbits . The breathing of the occupant of the chamber is therefore accurately reflected by small changes in pressure in the chamber. To precisely measure tidal volume the inlet and outlet of the chamber were closed for a few seconds . The breathing pattern of four human subjects was measured on four separate days, using an ultrasonic pneumotachograph (FIP Instruments, Field Road, Huntingdon, Cambridge). One hundred consecutive breaths were measured in terms of their inspiratory duration (t_I) expiratory duration (t_E) and tidal volume (V_T).

Variability of pattern was calculated as components of variance using the commercial analysis program SAS (SAS Institute Inc., SAS Circle,Box 8000 Cary,NC 27512-8000). We calculate that to detect with 80% certainty a 10% change in the mean values of inspiration, expiration and tidal volume with the experimental protocol and species we have used would require twelve men, nineteen rabbits or eight rats respectively.

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Publication 64.

Pirie,L & Davies,A.(1996)

Pulmonary receptors in the spontaneously breathing anaesthetised rat.

J.Physiol.495,158p,

Journal of Physiology (1995) 483.P

Pulmonary receptors in the spontaneously breathing anaesthetized rat

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It has been suggested that the rate of adaption of pulmonary stretch receptors should be greater in species with high respiratory rates to maintain effective reflex control of breathing (Bartlett & St John, 1979). Our alternative suggestion is that a greater proportion of rapidly adapting receptors exists in more rapidly breathing species. This increases the overall rate of adaption. To test this hypothesis the activity of seventy-three pulmonary mechanoreceptors with afferent fibres in the left vagus nerve was studied in sixteen spontaneously breathing anaesthetized (6 ml kg^{-1} 25% Urethane, I.P.) rats, during eupnoea and sustained inflation of the lungs. Fifty-one receptors discharged mainly in inspiration, and were slowly adapting (PSRs). Twelve discharged exclusively during early inspiration. Thirty discharged throughout inspiration and in early expiration. Nine discharged throughout inspiration and expiration.

Twenty-two rapidly adapting receptors (RARs) were spontaneously active during eupnoea (peak frequency $87{\cdot}52 \pm 6{\cdot}10$ Hz, mean frequency $28{\cdot}77 \pm 1{\cdot}25$ Hz), discharged almost exclusively during expiration ($2{\cdot}59 \pm 0{\cdot}23$ impulses in inspiration, $13{\cdot}34 \pm 0{\cdot}55$ impulses in expiration, 110 breaths) and, by definition, totally adapted in 0·25 s.

The patterns and total numbers of discharges for RARs were remarkably constant from breath to breath for individual receptors (e.g. $12{\cdot}4 \pm 0{\cdot}4$ impulses in five consecutive expirations).

The discharge frequencies of these receptors were comparable with those of other species (Widdicombe, 1954). The abundance of RARs was greater than in larger species (Roumy & Leitner, 1980).

The high proportion of RARs may represent an evolutionary advantage in neural control of the high frequency breathing of small mammals.

(Figures are means and standard errors of the mean).

Supported by the Norman Salvesen Emphysema Research Trust.

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Publication 65.

Davies,A. & Moores,C.(1996)

Oral endotracheal intubation of rabbits

Laboratory Animals, 30,182-183.

Oral endotracheal intubation of rabbits (*Oryctolagus cuniiculus*)

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Summary

Endotracheal intubation of rabbits is reported, both personally and in the literature, to be so difficult that special equipment has been constructed by other workers to facilitate the procedure. We report that the positioning of the operator, behind the animal, viewing from the dorsal surface of the head, facilitates this procedure enormously.

Keywords Endotracheal intubation; rabbits; *Oryctolagus cuniiculus*

To begin a study involving insufflation of drugs into the lungs of anaesthetized rabbits we required a method of endotracheal intubation which was quick, not traumatic to the rabbit's larynx and airway and which could be performed with certainty. All these requirements were directed to causing the least possible physiological stress and distress to the rabbits thus improving their welfare.

Tracheal intubation of the rabbit has been reported to us personally, and in the literature (Davis & Malinin 1974, Hoge *et al.* 1969, Schuyt & Leene 1977) as being difficult because of the mobility and anatomy of the larynx and upper airways. Special equipment has been designed to facilitate intubation (Schuyt *et al.* 1978) and a method relying on breath-sounds has been described (Alexander & Clark 1980). Ingenious methods, such as that of Kruger, Zellar & Schottmann (1994) demonstrate their considerable skill. We report here that the positioning of the operator is the most important determinant of success, and our finding that with correct positioning a difficult procedure became quick, easy and reliable.

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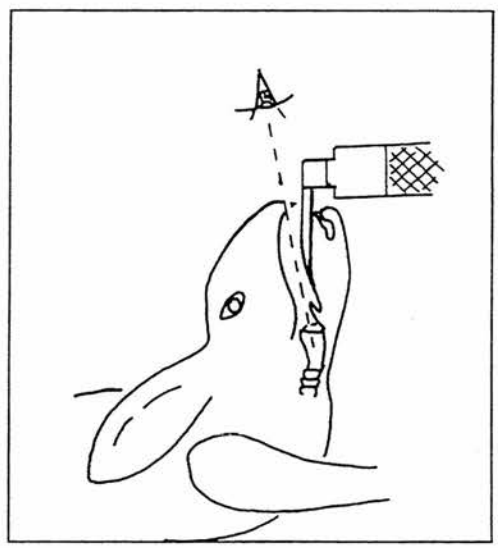

Fig 1 Visualization of the vocal cords

Materials and methods

Intubations have been performed on New Zealand White rabbits weighing 2.0–2.5 kg. They were anaesthetized via the marginal ear vein with Propofol (Zeneca) to a sufficient depth to allow the mouth to be easily opened.

The rabbit was laid prone on a table with its head close to a corner and its body extended close to one edge of the table; so operator and assistant stood facing each other with the assistant in front of the rabbit and the operator behind and to one side of the animal. The rabbit's head was tipped back and supported at the angle of the jaw by the assistant who at the same time gently pulled the tongue out of one side of the mouth.

With the operator standing *behind* the rabbit a Wisconsin laryngoscope with paediatric blade Number 1 was inserted and by viewing from behind the dorsal surface of the head the vocal cords could be very well visualized (Fig 1).

A sterile endotracheal tube O.D.3.5 mm (Portex Ltd, Hythe, Kent CT21 6JL) lubricated with a water-soluble sterile lubricant (K-Y Jelly, Johnson & Johnson Ltd, Slough, UK) could easily be inserted from this position, even without the necessity of completely obtunding laryngeal reflexes. After insufflation of drugs the tube was withdrawn. Recovery was rapid and within 5 min all rabbits had regained their righting reflexes and were moving calmly about their cages. We have experienced no complications arising from this method of intubation.

Discussion

We have previously experienced considerable difficulties intubating rabbits. We understand this is a common experience. We recommend the method described as completely reliable, without trauma to the rabbits and without subsequent adverse effects.

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Publication 66.

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Pattern of breathing in a rabbit and a rat model of emphysema.

J.Physiol.495,158p.

Journal of Physiology (1996) **495.P**

Pattern of breathing in a rabbit and a rat model of emphysema

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The pattern of breathing parameters (T_I, T_e, V_T)* of conscious control and emphysematous Dutch rabbits and rats was measured using the whole body plethysmography method of Bartlett & Tenney (1970), which was developed from the method of Drorbaugh & Fenn (1955).

Our previous study (Dallak *et al.* 1995) showed that to detect a 10% change in the breathing pattern parameters at 80% power, it is necessary to measure twenty breaths per day for 4 days. The number of Dutch rabbits required to detect a 10% change in T_I, T_e and V_T is 10, 19 and 3 and that of rats is 4, 8 and 6.

Emphysema was induced in six anaesthetized Dutch rabbits (under Hypnorm 0·4 ml kg^{-1} I.M., Diazepam 1 mg kg^{-1} I.V. and Butorphanol 0·1 mg kg^{-1} I.V. as a reversal agent) by giving type 4 pancreatic elastase (240 units kg^{-1} in 1 ml sterile 0·9% saline) by insufflation and in ten rats (under 2% Halothane anaesthesia) using papain (120 mg kg^{-1} in 0·25 ml 0·9% saline). The pattern of breathing of the rabbits was measured before induction of emphysema and 4 weeks after, and that of the rats was measured before and 2 weeks after induction. The pattern of breathing parameters (means ± S.D.) before and after induction of emphysema was as follows:

In rabbits: T_I, 0·54 ± 0·24, 0·42 ± 0·11, n.s.; T_e, 0·67 ± 0·20, 0·50 ± 0·13, n.s.; V_T, 13·3 ± 2·9, 12·6 ± 3·1, n.s.

In rats: T_I, 0·21 ± 0·02, 0·22 ± 0·02, n.s.; T_e, 0·31 ± 0·06, 0·40 ± 0·07†; V_T, 2·02 ± 0·30, 2·45 ± 0·30† († $P < 0·01$, Wilcoxon signed-rank test).

The results show that the induction of emphysema in the rabbits did not significantly alter the pattern of breathing parameters, while the induction of emphysema in rats made breathing deeper and slower.

Thus changes in the parameters describing the pattern of breathing following induction of emphysema are more readily detected in the papain rat model than in the elastase rabbit model.

* T_I is inspiration time in seconds, T_e is expiration time in seconds and V_T is tidal volume in millilitres.

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Adaptation of pulmonary receptors in the spontaneously breathing anaesthetized rat

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Adaptation of pulmonary receptors in the spontaneously breathing anaesthetized rat
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ABSTRACT: It has been suggested that species with high breathing frequencies have pulmonary stretch receptors which adapt more rapidly than species with low breathing frequencies. This has proved not to be so. Our hypothesis is that this theory is in fact correct if modified so that overall rate of adaptation of afferent vagal activity, *i.e.* the sum of stretch and rapidly adapting receptors, is considered. A rapidly breathing species, such as the rat, would thus have a greater proportion of rapidly adapting receptors, than a more slowly breathing species.

To test this hypothesis, we measured the proportion of rapidly adapting pulmonary mechanoreceptors in spontaneously breathing rats for comparison with existing results from more slowly breathing species.

We found there to be one rapidly adapting receptor for every three slowly adapting receptors present. This measurement has not previously been made in spontaneously breathing rats. The ratio of rapidly to slowly adapting pulmonary receptors in the species sequence cat-rabbit-rat is the same as the ratio of their breathing frequencies (3:4:10).

We propose that the difference in proportion of slowly to rapidly adapting pulmonary receptors in different species may be related to their eupnoeic breathing frequency.
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BARTLETT and ST JOHN [1] made the important observation that adaptation of pulmonary mechanoreceptors imbues them with dynamic as well as static response characteristics. It would, thus, seem important to consider the degree of adaptation that takes place in a time interval similar to the animal's respiratory frequency. BARTLETT and ST JOHN [1] postulated that pulmonary stretch receptors (PSRs) of species with different eupnoeic frequencies would have different rates of adaptation to accurately signal lung conditions. This did not prove to be the case from their results. However, their observations were restricted to pulmonary stretch receptors.

Rapidly adapting receptors (RARs), sometimes called "irritant" or "deflation" receptors, are found in the lungs of many species [2]. It is possible that a change in the proportion of these receptors, relative to the number of PSRs, provides the different degree of adaptation required. The important concept of a link between receptor adaptation rate and frequency of eupnoeic breathing is linked to the role of PSRs and RARs in control of breathing. It is generally accepted that PSRs terminate inspiration and extend expiration. We have demonstrated [3] that RARs terminate expiration, and hence can profoundly affect breathing frequency. We have excluded C-fibre receptors from the present study because there is, as yet, insufficient quantitative description of their activity to enable between species comparison to be made.

Two recent publications [4, 5] have reported the activity of pulmonary receptors in anaesthetized, paralysed, open- and closed-chested rats, ventilated by positive pressure. These authors report very little RAR activity. If this were true for intact spontaneously breathing rats, it would oppose the theory that small mammals with high respiratory rates have a higher proportion of rapidly adapting lung receptors.

We have used the rat as a model of respiratory control in human lung disease [6]. If the rat is to be a useful model in this context, it is important to know whether results obtained can be compared with those obtained in cats dogs and rabbits [3, 7, 8].

There is, as yet, insufficient evidence from a wide variety of species to give a categorical answer to the question of whether RAR have the same function in rats as in other species. However, drawing a parallel with PSRs, the Hering Breuer inflation reflex in all species, although varying in strength between species, is in all cases attributed to PSRs. Also, the way in which the number of receptors of a certain type in a species is determined needs to be considered and depends on the definition used. The overlap of the conduction velocities in fibres from PSRs and RARs tends to make categorization by this criterion difficult. However, these differences in definition only become important when considering subgroups of RARs and PSRs. In our experience with rabbits [3] and rats, the functional difference is unambiguous.

To determine whether the reported absence of RARs in rats was due to the nature of the preparation or a true species difference, and to measure the proportion of RARs

present, we recorded the activity of pulmonary receptors in closed-chested, spontaneously breathing rats during eupnoea, and during activity provoked by inflation of the lungs.

Methods

Animals and preparation

Fifteen barrier reared Sprague-Dawley rats, weighing 564±21.4 g, were anaesthetized with an intraperitoneal injection of 1.5 $g \cdot kg^{-1}$ urethane as a 25% solution, supplemented as necessary *via* a catheter in the left femoral vein. A short tracheal cannula was inserted and airflow recorded by a Fleisch pneumotachograph head and Mercury CS5 differential pressure transducer. The left vagus nerve was cut high in the neck and the distal cut end placed in a copper tray filled with liquid paraffin. "Single fibre" preparations were made from strands of nerve that displayed respiratory rhythm when placed on a pair of silver wire electrodes. Carbon dioxide in the respired air was monitored by a Beckman L.B.1 gas analyser.

Experimental method

A period of eupnoeic breathing was recorded. The rat's lungs were then inflated four times with 0.5 and 1 kPa airway pressure. Three minutes separated the inflations, which were administered by the method of DAVIES and ROUMY [3]. This consisted of having a solenoid operated valve very rapidly connect the tracheal cannula from the atmosphere to a 50 L drum maintained at the required pressure. Inflation was synchronized with the peak of inspiration as detected by zero flow. Positive pressure was maintained until the rat took a spontaneous inspiration.

Recording

Electrical activity of the single fibre preparations was amplified by a high-gain RC amplifier (Neurolog), fed to an audio-amplifier and loudspeaker and recorded directly, and as transistor-transistor logic (TTL) pulses, together with the other physiological variables, by a TEAC XR-30 recorder.

Records were taken for four breaths before applying the step in pressure, and for 2–3 s after the first inspiratory effort.

Analysis

Analysis was undertaken "off-line" by digitizing the tape records *via* a Cambridge Electronics Design 1401 converter; and using a modified proprietary computer analysis program (Cambridge Electronics Design Spike 2).

Receptors were classified as slowly or rapidly adapting. Conduction velocity was initially used to differentiate between slowly and rapidly adapting receptors, but it soon became apparent that the difference in response to a step of inflation was sufficient to clearly differentiate between the two types. Adaptation was quantified using a form of KNOWLTON and LARRABEE [7] Adaptation Index, modified to provide criteria which enabled a clear differentiation between slowly and rapidly adapting receptors, without the profound physiological interventions of paralysis or thoracotomy. Which would also have frustrated the objective of recording under eupnoeic conditions.

The number of action potentials in the third 0.25 s of lung inflation with a pressure of 1 kPa, was subtracted from the number in the first 0.25 s, and the result expressed as a percentage of the number of action potentials in the first 0.25 s of inflation. This expression of adaptation was termed "Adaptation Index", and clearly distinguished between slowly and rapidly adapting receptors. It also involved a time interval appropriate to the rat's breathing frequency. Rapidly adapting receptors were defined as having an index of 100%.

Occasionally, C-fibres, identified by their low conduction velocity, were isolated and discarded. Not all receptors, which could be clearly categorized manually as rapidly or slowly adapting, were suitable for computer analysis during inflation. The most common artifact defeating computer analysis was a second fibre discharging during inflation, which was not detected during recording.

Statistics

Statistical significance of difference between mean values, (shown as mean±SEM), calculated by Student's unpaired t-test was taken as a p-value less than 0.05. To ensure the maximum rigour, the mean values obtained for individual receptors were used for comparison.

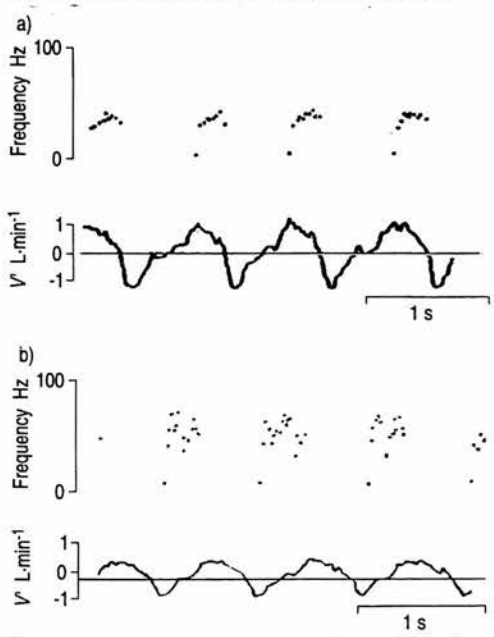

Fig. 1. – Computer record of respiratory airflow (expiration downward) and instantaneous frequency of discharge of: a) Type I PSR; b) expiratory RAR, in two rats. $\dot{V}$: airflow; PSR: pulmonary stretch receptor; RAR: rapidly adapting receptor.

Results

Of the 85 receptors recorded, 12 were inadequate for later analysis. The 73 receptors characterized in this study could be divided into PSR (fig. 1a) and RAR (fig. 1b) on the criterion of the Adaptation Index described in the Methods section. All had the characteristics of receptors with myelinated fibres, when their discharge was inspected on an oscilloscope. Five fibres with discharge characteristics associated with unmyelinated fibres were abandoned.

In two rats, conduction velocity was measured in fibres firing in inspiration and seen to be slowly adapting (36.1± 7.1·ms^{-1}; n=8) and those firing in expiration which were rapidly adapting (14.6±4.6·ms^{-1}; n=4).

The PSRs were divided into three types; and of these, two types, I and II, were very similar as described below. The mean adaptation index of PSR was 42.7±3.9%. The mean adaptation indices of the three individual types which will be described were: type I 58.0±8.8%; type II 42.6±1.8%; and type III 27.5±3.8%. Rapidly adapting receptors had an index of 100%.

Slowly adapting receptors (PSRs)

The receptors categorised as PSRs were further divided into three types on the basis of their discharge pattern during an eupnoeic respiratory cycle of mean tidal volume 2.83±0.20 mL.

Type I (16% of all receptors). These 12 receptors discharged almost exclusively during mid and late inspiration as shown in figure 2. The small number of spikes (table 1a) occurring during expiration were restricted to the first 15% of expiratory duration (t_E). Their peak, mean and minimum frequencies of discharge during eupnoea with a tidal volume (V_T) of 2.83±0.20 mL and their frequencies on lung inflation (0.5 and 1.0 kPa) are shown in tables 1a and 2.

Type II (41% of all receptors). These 30 receptors were placed in a separate category from Type I because of their significantly higher discharge frequency (table 1a).

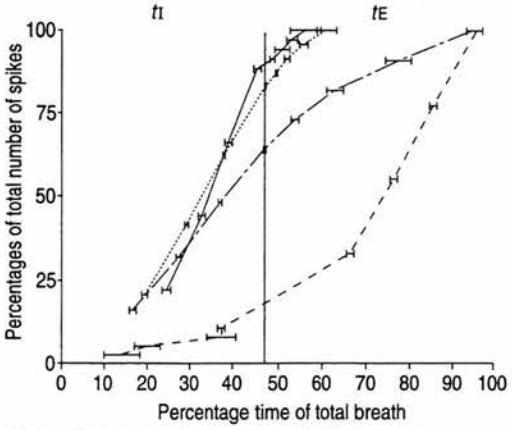

Fig. 2. – Distribution of the discharge of PSR Type I (12 receptors) PSR Type II (30 receptors), PSR Type III (9 receptors), and expiratory RARs (22) throughout a breath. The means±SEM of percentage of the total number of action potentials occurring at times through the respiratory cycle are shown. ——: Type I; ------: Type II; – – – -: Type III; - - - -: RAR. t_I: inspiratory duration; t_E: expiratory duration. For further abbreviations see legend to figure 1.

During expiration, their discharge was restricted to the first quarter of t_E. Thus, these receptors discharged over a somewhat greater range of the breathing cycle than Type I receptors. They were also placed in a separate category because of their lower adaptation index and significantly higher discharge frequency. Their response to eupnoea and sustained lung inflation is shown in tables 1a and 2.

Type III (12% of all receptors). These nine receptors differed more clearly from Type I and II than did Type I and II from each other. They discharged throughout inspiration and into expiration, as shown in figure 2. Their response to eupnoeic breathing and sustained lung inflation is shown in tables 1a and 2.

The only purpose of dividing the PSRs into Types I, II and III was to compare them with types reported by

Table. 1. – Discharge characteristics of: a) pulmonary slowly adapting receptors and; b) rapidly adapting receptors

Receptor type	Phase	Receptors n	Peak frequency Hz	Mean frequency Hz	Minimum frequency Hz	Impulses in phase
a) Pulmonary slowly adapting receptors						
PSR I	t_I	12	45.8±5.2	20.4±2.4	25.7±2.4	8.3±0.82
	t_E	12	5.4±3.7	1.79±0.42	6.4±2.5	1.08±0.15
PSR II	t_I	30	89.8±3.7	62.7±3.6	41.6±1.3	24.7±1.2
	t_E	30	67.6±7.5	10.9±1.2	34.3±2.3	5.0±0.45
PSR III	t_I	9	114.5±10.1	74.5±7.3	44.8±4.7	30.2±2.7
	t_E	9	92.2±8.0	32.0±5.2	17.3±4.2	16.7±2.8
Mean all PSR	t_I	51	86.1±4.4	57.4±3.8	40.0±1.6	22.4±1.4
	t_E	51	57.1±5.1	13.2±2.0	25.7±2.3	6.0±0.99
b) Rapidly adapting receptors						
RAR	t_I	22	18.5±3.0	5.0±1.3	7.7±1.2	1.7±0.48
	t_E	22	87.5±10.4	28.8±3.0	18.1±2.3	13.6±1.4

Mean±SEM of peak and minimum frequency of discharge (Hz) of the four receptor types, measured from interspike interval. Mean frequency in this table = total spikes in the phase/duration of phase, and therefore can be less than minimum frequency, calculated from interspike interval. Frequencies of discharge of Types II and III receptors are significantly different ($p<0.05$) from the mean of Type I receptors. For other criteria used to differentiate between receptor types see text. t_I: inspiratory duration; t_E: expiratory duration; PSR: pulmonary stretch receptor; RAR: rapidly adapting receptor.

Table 2. – Adaptation to inflation

Receptor type	Receptors n	Time from onset of inflation s	Impulses n 0.5 kPa	1.0 kPa
I	12	1st 0.25	15.8±1.2	19.2±2.3
		3rd 0.25	3.4±1.4	8.7±2.2
II	30	1st 0.25	25.5±2.1	31.6±4.1
		3rd 0.25	8.9±1.7	18.3±2.6
III	9	1st 0.25	31.0±2.1	31.7±2.2
		3rd 0.25	16.3±1.3	21.5±1.5

Mean±SEM of number of impulses (action potentials) in 1st and 3rd 0.25 s of lung inflations by 0.5 and 1.0 kPa for inspiratory receptor types I, II and III. Expiratory, rapidly adating receptors, totally adapted within 0.25 s. The adaptation indices of Type I and Type II receptors were not significantly different. The adaptation index of Type III receptors was significantly different ($p<0.01$) from the other two types.

other workers (see Discussion). The pooled values of discharge properties of all the PSRs are, therefore, given in table 1a for comparison with those of the RARs, which was the main purpose of this investigation.

Rapidly adapting receptors (RARs)

These 22 receptors (30% of all receptors) discharged almost exclusively during expiration in eupnoeic breathing, as shown in figure 2 and table 1b.

Because "mean frequency" was calculated by dividing number of spikes by duration of inspiration (tI) or expiration (tE) it can, apparently paradoxically, be less than "minimum frequency", calculated from interspike interval.

Consistency of discharge. RARs have previously been described as irregular in their discharge. RARs in the rats used for these experiments discharged with a highly consistent pattern. Figure 3 shows the timing of 25, 50 and 100% of total discharge of two typical RARs over five consecutive breaths. Table 1b shows the discharge characteristics of 22 RARs during eupnoea.

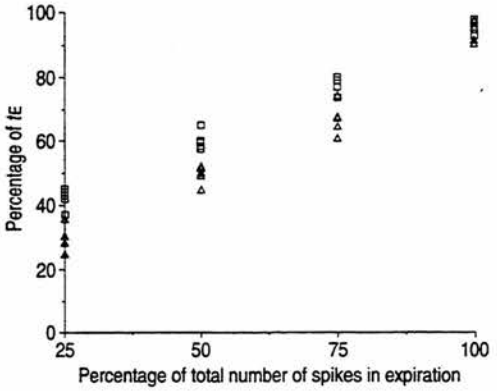

Fig. 3. – The regular nature of discharge of rapidly adapting receptors in rats, demonstrated by plotting percentage of duration of expiration (tE) against percentage total number of spikes in expiration for five consecutive breaths for two typical rapidly adapting receptors (RARs). △ : receptor 1; □ : receptor 2.

Discussion

BARTLETT and ST JOHN [1] postulated that pulmonary mechanoreceptors of animals with different eupnoeic breathing frequencies would have different rates of adaptation. This did not prove to be the case from their results. However, their observations were restricted to PSRs. Rapidly adapting receptors, sometimes called "irritant" or "deflation" receptors, are found in the lungs of many species. It may be that a change in the proportion of RARs provides the different overall degree of adaptation required by different species [9]. Information concerning rapidly adapting receptors exists for a number of species [3, 10, 11]. No information has previously been available for rats. The association of adaptation rate with frequency of breathing receives support from our earlier findings [3] that the activity of RARs profoundly affects expiration, accelerating breathing.

In considering the control of pattern of breathing, it is important to consider the degree of receptor adaptation that takes place in a time interval approximately similar to the animal's respiratory cycle. The classical Adaptation Index of KNOWLTON and LARRABEE [7] involves a 2 s lung inflation. A rat might take three breaths in that time. WIDDICOMBE [10] points out that such an Adaptation Index "does not distinguish between adaptation rates of endings which cease firing within 1 s, so that stimuli well above threshold must be used and the endings must discharge for 2 s or more". To overcome these difficulties, we measured the degree of adaptation in 0.75 s of the application of a step stimulus.

Measured in the conventional open-chested preparation, rats have PSRs with adaptation indices similar to those of other species [4]. Our measure of adaptation is a more rigorous test of whether a receptor is rapidly or slowly adapting than conventional definitions. Recent publications [4, 5] have pointed out the paucity of information about the properties of pulmonary receptors in the rat and described discharge patterns mainly under conditions of respiratory paralysis and artificial ventilation.

We report here receptor activity measured without resort to the paralysis or thoracotomy of previous investigations, which would exclude recording under eupnoeic conditions. The difference between PSRs and RARs was very clear, and did not depend on our tentative division of PSRs into three types, undertaken for comparison with other published findings. Our findings are sufficiently similar to those reported previously to bear comparison, but differ in a number of important ways. Many differences may arise from the differences in transmural pressure found in the open- or closed-chested rats. That opening the chest affects receptor activity is clearly demonstrated in the publications by TSUBON [5] and BERGREN and PETERSON [4]. In view of the profound differences between open- and closed-chested preparations, the properties of the PSRs in the report by BERGREN and PETERSON [4] and in our results are remarkably similar. We found 24% PSRs (16% of all receptors) to be exclusively inspiratory (Bergren and Peterson - 25%). Fifty nine of our PSRs (41% of all receptors) discharged throughout

inspiration and early expiration (Bergren and Peterson - 49%). Eighteen percent of our PSRs (12% of all receptors) discharged throughout the respiratory cycle.

As spontaneously breathing rats have respiratory frequencies of the order of 100 breaths·min^{-1} [12] with virtually no expiratory pause, the "deflationary (D) slowly adapting receptors (SARs)" of BERGREN and PETERSON [4], which made up 18% of their SAR (PSR) population, and the "deflation sensitive receptors" of TSUBONE [5], both of which were stimulated during the deflationary phase of their ventilating pump, have little equivalence to any of our receptors. One may speculate that these receptors would approximate more closely to one of the groups that we describe, if the rats from which they were recorded were breathing spontaneously rather than paralysed and ventilated by positive pressure.

Table 1 shows peak and minimum frequency derived from interspike intervals. Mean frequency is the number of action potentials in a phase of breathing (inspiration (I) or expiration (E)) divided by the duration of that phase, and can therefore be less than minimum frequency. Figure 2 shows the phase-spanning nature of the Type III receptors, and that Type I and RAR discharges are highly polarized into inspiration and expiration, respectively. The properties of Type I and Type II receptors are very similar. We have tentatively separated them into two types, mainly on the basis of adaptation rates and eupnoeic frequencies of discharge. WIDDICOMBE [10] comments "adaptation rate alone does not distinguish between different groups of pulmonary sense organs". It may well be that further investigation will not sustain this separation, which is not central to the thesis being tested by this study.

Because the imposed ventilatory cycles of paralysed rats used by other workers was so different from the pattern of spontaneous breathing of our rats, it is difficult to make comparisons of frequency of discharge. It can be said that the total number of impulses produced by Type I PSRs, in a respiratory cycle of inspiratory duration 0.39±0.005 s and expiratory duration 0.43±0.013 s (n=60 breaths; 12 rats) in our study, is of the same order of magnitude as all except the "mostly inspiratory" receptors reported by BERGREN and PETERSON [4] during a pump cycle of approximately 0.9 s; of which approximately 0.2 s was occupied by inflation. As most of the activity reported by these authors took place during inflation, there is an approximation to the rate of discharge that we report. SCHOENER and FRANKEL [13], who used a realistic frequency (2 Hz) to ventilate their paralysed rats, reported a mean discharge frequency of 96±7 Hz for PSRs. This compares with our overall mean frequency for PSRs of 70.0±1.7 impulses·s^{-1} (table 1).

The algorithm that we used to measure adaptation rates of our receptors addressed the problems of matching the period investigated to a physiological breathing pattern, and the criticism by WIDDICOMBE [10] of the problem of attributing an adaptation index to receptors which silenced within 1 s of applying inflation. Our form of index also addresses the problem of using "peak frequency" in calculating adaptation index. Peak frequency, by definition, measures the time interval between only two action potentials.

Our longer, albeit very brief, interval provides a more representative sample of receptor activity on inflation. Our adaptation index distinguished clearly between the three types of PSRs and RARs.

Pulmonary stretch receptors respond to the degree and rate of change of volume of the lungs [14], and have been categorized into those that saturate above 1 kPa transmural pressure and those with a more linear response. Some workers report [15, 16] that there is a continuum rather than discrete PSR types. Whilst this may be true for Type I and II PSRs found in our rats, these were very different in rate of adaptation and position of firing in the respiratory cycle from Type III PSRs or RARs (fig. 2 and tables 1 and 2). No attempt was made to identify the location of these receptors. Some may have been extrathoracic being active during expiratory flow [17].

In our rats, 30% of receptors active during spontaneous breathing were rapidly adapting. This is in direct contrast to BERGREN and PETERSON [4] who found only 7% of their receptors were rapidly adapting. TSUBONE [5], on the other hand, using an open-chested paralysed preparation like BERGREN and PETERSON [4], found "irritant-like receptors", which discharged during both inflation and deflation. The only apparent difference between the methods of these two studies was the use by Bergren and Peterson of a 0.3–0.5 kPa end-expiratory pressure and the repeated use of exposures of 5–20s to dimethyl-ether vapour to silence PSRs. Ether vapour stimulates RARs in guinea-pigs [18]. However, concentrations of 7.5–14.5% inhibits RARs [10]. Because of their more central and superficial position in the airways compared with PSRs [19], it is likely that the RARs in the study by BERGREN and PETERSON [4] received higher concentrations of the vapour used repeatedly to silence PSRs than the PSRs themselves. We cannot say whether such treatment permanently silences RARs, but it may explain the difference between the results of the latter study and our findings. It might also be that the use of end-expiratory pressure prevented atelectasis, which would have caused increased activity in the RARs of Tsubone's preparation compared to those of Bergren & Peterson.

There is a fundamental problem when comparing lung receptor activity of open-chested animals, ventilated by positive pressure, with the more physiologically normal spontaneously breathing animal. In the open-chested animal, the airways are expanded by a pressure which compresses the innermost epithelial layers against the underlying muscle. An important property of bronchial smooth muscle is that it becomes noncompliant at relatively low transpulmonary pressures (around 1 kPa [20]). Conversely, in normal, closed-chested, spontaneously breathing animals the innermost epithelial layers are placed in a state of tension by expansion of the lung. There are no data yet available as to whether these differences activate receptors in different ways; but it could be expected that RARs, being found in the innermost lining layers, would show greater differences than PSRs located within the muscle wall. It may be that the different patterns of RAR activity, in particular the presence of irregular activity during positive-pressure inflation ([8] for example) are, in part, a result of this effect.

In conclusion, we maintain that rapidly adapting receptors do exist in considerable numbers in rats and are active during spontaneous ventilation. They are present in the ratio of approximately 1 rapidly adapting recep-

tor to 3 pulmonary stretch receptors. This compares with ratios of 1:4 in the rabbit [11], and 1:10 in cats [10]. The breath durations of adults of these species is also in the ratio 3:4:10 [9]. This supports our suggestion (a modification of that of BARTLETT and ST JOHN [1]) that the respiratory frequency of a species is related to the overall adaptation rate of all its pulmonary receptors.

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Publication 68.

Moores,C.,Davies,A. & Dallak,M.(1997)

Sevoflurane has less effect than halothane on pulmonary afferents.

British Journal of Anaesthesia, 80, 257-259.

British Journal of Anaesthesia 1998; 80: 257–259

Sevoflurane has less effect than halothane on pulmonary afferent activity in the rabbit†

C. MOORES, A. S. DAVIES AND M. DALLAK

Summary

We have compared the effects of sevoflurane and halothane on the discharge frequencies of 19 slowly adapting and four rapidly adapting lung receptors in the rabbit by recording from single vagal fibres. Both agents reduced the discharge frequency of slowly adapting receptors during expiration ($P<0.0005$), halothane having a greater effect than sevoflurane ($P<0.0005$). Neither agent had any effect on discharge frequency at the end of inspiration when discharge frequency is at a maximum. Neither agent affected the discharge frequency of rapidly adapting receptors. (*Br. J. Anaesth.* 1998; **80**: 257–259)

Keywords: anaesthetics volatile, sevoflurane; anaesthetics volatile, halothane; lung, receptors; rabbit

Lung receptors include slowly adapting (stretch) receptors (SAR), rapidly adapting (irritant) receptors (RAR) and J receptors. They form the afferent limbs of airway reflexes, including the Hering–Breuer reflex, reflex bronchoconstriction and the cough reflex.

The activity of these lung receptors has been shown to be influenced by volatile anaesthetic agents such as chloroform, ether,[1] halothane, isoflurane and enflurane.[2] Nishino, Anderson and Sant'Ambrogio found that the modern agents increased the firing threshold of SAR while at the same time increasing their discharge frequency above this threshold. They also found that increasing concentrations of volatile agents decreased the activity of RAR. They could not demonstrate a quantitative difference between the agents on either receptor type.[2]

To date, sevoflurane has not been studied. It differs from other volatile agents in that it appears to be much less irritating to the airway, being associated with a lower incidence of airway-protective reflexes.[3] It seems likely, therefore, that sevoflurane may also have a different effect on intrapulmonary receptors. In this study, we compared the effect of halothane and sevoflurane on the activity of intrapulmonary receptors measured from single vagal fibres in rabbits undergoing ventilation.

Methods and results

After obtaining approval from the Home Office, we studied four New Zealand White rabbits. Each was anaesthetized with 25% urethane 7 ml kg^{-1}. The femoral vein was cannulated to allow i.v. injections, and the femoral artery was cannulated for measurement of arterial pressure and heart rate. The trachea was cannulated, the rabbit paralysed with vecuronium 0.2 mg kg^{-1} and its lungs ventilated with air using a tidal volume of 6 ml kg^{-1} and a ventilatory frequency of 80 bpm to maintain end-tidal carbon dioxide partial pressure close to the level measured before ventilation. Airway flow was measured using a pneumotachograph, the flow signal being integrated electronically to give volume (CS5, Griffin). End-tidal concentrations of anaesthetic gas and carbon dioxide were measured continually (Capnomac Ultima, Datex).

Both vagi were divided in the neck to prevent reflex bronchoconstriction which may influence SAR activity. The distal end of the left vagus was desheathed and divided into thin filaments. Recordings were made from these filaments using bipolar platinum electrodes and nerve discharges were displayed on an oscilloscope and fed to a loudspeaker. Further subdivision of each filament continued, until a filament was found containing a single active nerve fibre from a pulmonary afferent. The single fibre was identified as originating from either a pulmonary stretch receptor or rapidly adapting receptor on the basis of its pattern of firing during the respiratory cycle and its response to short pulses of either positive or negative pressure.

The lungs were ventilated with oxygen for 2 min and then either sevoflurane or halothane was introduced at a concentration of 2% for 1 min followed by 5% for 1 min. The rabbit's lungs were then ventilated with oxygen for 2 min. Thereafter, 2% and 5% of the second agent was introduced as for the first. The vagal filament was then discarded, another filament was dissected from the vagus and the procedure repeated. The order in which the volatile agents were used was reversed in consecutive procedures.

Nerve impulses were converted into 5-V electronic pulses using a windows discriminator (Neurolog, Digitimer Ltd). Airway flow and pressure, tidal

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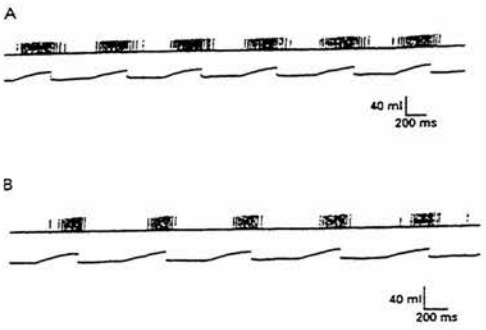

Figure 1 A: Slowly adapting receptor activity and inspired volume during control conditions. The upper trace shows 5-V processed nerve impulses and the lower trace inspired volume (integrated flow signal). Receptor activity increased with increasing lung volume and decreased to a minimum during expiration. B: Impulses from the same receptor exposed to 5% halothane. The threshold at which firing commenced was higher, but the maximum rate of firing was unchanged.

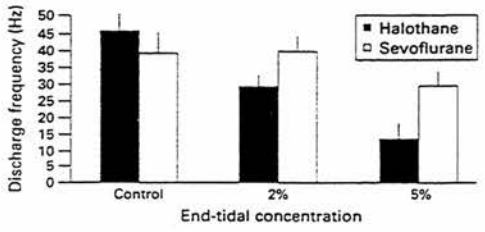

Figure 2 Mean (SEM) discharge frequency of slowly adapting receptors during expiration. Concentration effects and difference between agents were significant ($P<0.0005$).

volume, arterial pressure and nerve impulses were recorded onto eight-track magnetic tape (XR30, TEAC). Statistical analysis was performed using repeated measures ANOVA (SPSS for Windows.)

Recordings were made from 23 single fibres: 19 from SAR and four from RAR.

SLOWLY ADAPTING RECEPTORS

Figure 1A shows the characteristic discharge pattern of an SAR during the respiratory cycle. With increasing lung volume the discharge frequency of the receptor increases. Figure 1B illustrates the discharge pattern of the same receptor during administration of 5% halothane. This receptor ceases to discharge for a period during expiration; however, some receptors discharged throughout the respiratory cycle, and therefore separate analysis of discharge frequency during inspiration and expiration was necessary, rather than analysis of the duration of discharge of the receptors.

Expiration

During expiration, the effect of both halothane and sevoflurane is to reduce the receptor discharge frequency. Figure 2 demonstrates the effect of increasing concentration on receptor discharge frequency during expiration, when discharge frequency is at a minimum. The decrease in discharge frequency with increasing concentration was significant ($P<0.0005$). Halothane had a significantly greater effect than sevoflurane in reducing discharge frequency during expiration ($P<0.0005$).

Inspiration

During inspiration, we were unable to demonstrate any effect of either agent on receptor discharge frequency. Mean discharge frequency during the final 100 ms of inspiration for halothane was 107.7 (SEM 6.5) Hz (control) and 106.7 (7.2) Hz (5% end-tidal concentration), and for sevoflurane 103.5 (7.0) Hz (control) and 114.5 (7.1) Hz (5% end-tidal concentration).

RAPIDLY ADAPTING RECEPTORS

During the course of this investigation, we recorded from only four RAR as these receptors form a small proportion of the lung receptors in the rabbit. Measurements of nerve discharge frequency were averaged over a 5-s period. At the concentrations used, we found no significant effect of either agent on the discharge frequency of the RAR. Mean discharge frequency for halothane was 6.1 (SEM 1.9) Hz (control) and 6.7 (1.7) Hz (5% end-tidal concentration), and for sevoflurane 5.6 (1.9) Hz (control) and 7.7 (1.2) Hz (5% end-tidal concentration)

In the case of two receptors, we recorded airway pressure. There was no significant change in maximum airway pressure during ventilation with either volatile agent, suggesting that they did not cause a significant degree of bronchodilatation which may have altered SAR activity. Mean maximum airway pressure during ventilation for halothane was 1.39 (SEM 0.1) cm H_2O (control) and 1.49 (0.1) cm H_2O (5% end-tidal concentration), and for sevoflurane 1.39 (0.1) cm H_2O (control) and 1.44 (0.1) cm H_2O (5% end-tidal concentration).

Comment

We have shown that the predominant effect during expiration of halothane and sevoflurane is to decrease the discharge frequency of SAR. This represents an increase in the discharge threshold of the receptors, and confirms the findings of Nishino, Anderson and Sant'Ambrogio using halothane, isoflurane and enflurane.[2] Nishino, Anderson and Sant'Ambrogio did not demonstrate a significant quantitative difference between the effect of these agents on SAR; we found that halothane had a significantly greater effect than sevoflurane on the discharge frequency of SAR during expiration. Sevoflurane has less effect than other agents on upper airway receptors, as manifest by its low level of airway irritability, and it is interesting that it also had less of an effect on lung SAR.

We did not demonstrate an effect of either volatile agent on the discharge frequency of SAR at the end of inspiration when their firing rate is at a maximum. Other workers have found in decerebrate cats that the modern agents sensitize these receptors above their firing threshold.[2] It is possible that had we used larger

tidal volumes, applying a greater stimulus to these receptors, their discharge frequency at end-expiration may have been influenced by the volatile agents.

SAR form part of the Hering–Breuer inflation reflex which in animals is important for the control of breathing pattern.[4] Halothane and sevoflurane produce tachypnoea in animals and humans.[5,6] The increase in ventilatory frequency that is seen in animals during anaesthesia with halothane is smaller after vagotomy,[7] suggesting that changes in lung afferent activity are partly responsible presumably via the Hering–Breuer reflex. In both animals and humans, tachypnoea during anaesthesia with sevoflurane is less than that with halothane and vagotomy does not alter the increase in ventilatory frequency that sevoflurane causes in decerebrate cats.[7] It is possible that the differences between halothane and sevoflurane in animals may be caused by differences in the sensitivity of SAR to the two agents, although a central cause cannot be excluded. Although in anaesthetized humans there is little evidence that the Hering–Breuer reflex is important in modulating eupnoeic breathing,[8,9] the effect of volatile agents on lung receptors could possibly result in the modulation of other airway reflexes, such as the cough reflex, reflex bronchodilatation and bronchoconstriction.

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Publication 69.

Moores,C.,Davies,A. & Dallak,M.(1997)

The effect of halothane and sevoflurane on slowly adapting lung stretch receptors.

British Journal of Anaesthesia,78,454,

Effect of halothane and sevoflurane on slowly adapting lung receptors in the rabbit

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We compared the effect of halothane and sevoflurane on the discharge frequency of slowly adapting receptors (SARs) in the lungs of rabbits using a single nerve fibre recording technique.

Halothane, enflurane and isoflurane were studied in the past[1] and were found to raise the threshold of firing of lung SARs, while at the same time increasing their discharge frequency above this threshold. No quantitative difference was found between these agents. Sevoflurane has not been studied but is known to be much less irritant to the airways,[2] and therefore its effect on lung receptors may be different from other agents.

Four New Zealand White rabbits were studied. Each received 25% urethane 7 ml kg^{-1}, with vecuronium 0.2 mg kg^{-1} and its lungs were ventilated with a tidal volume of 6 ml kg^{-1} at a rate of 80 beat min^{-1}. Both vagi were divided and the left was desheathed and repeatedly split into thin filaments from which recordings were made, until a filament was found containing a single nerve fibre arising from a lung SAR. The lungs were then ventilated with oxygen containing sevoflurane or halothane at end-tidal concentrations of 2.0% followed by 5%. A number of receptors from each rabbit were studied. Recordings were made of tidal volume, airway flow and pressure, arterial pressure, heart rate and nerve fibre discharge frequency. Comparisons were made using two-factor repeated measures analysis of variance.

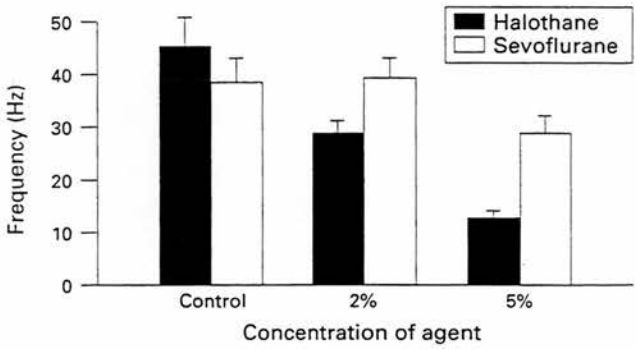

Figure 3 Discharge frequency during expiration of slowly adapting receptors exposed to volatile agents (mean (SEM)).

Recordings were made from 19 slowly adapting receptors. We found that increasing agent concentration significantly decreased the frequency of discharge of the receptors during expiration ($P<0.0005$) (fig. 3). The decrease caused by halothane was significantly larger than that caused by sevoflurane ($P<0.0005$). However, we found no significant effect of either agent on the discharge frequency of the receptors during the final 100 ms of inspiration, when discharge frequency is at a maximum.

Key words
Anaesthetics volatile, sevoflurane. Lungs, slowly adapting receptors. Rabbit.

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Publication 70.

Ponting,R.,A.I.Heusch. & Davies,A.(1999).

A fluid switch detector of air movements.

J.Physiol.Proc.Vol.515, 44p,

Establishment of a facility for transgenic rat production

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In many areas of physiology rats are established as the species of choice, and therefore the usefulness of transgenic mouse technology for answering physiological questions can be limited. Following the development of transgenesis in rats (Mullins *et al.* 1990), the application of this technology in the larger species has been sporadic. This is partly due to technical obstacles, including the difficulty of microinjecting DNA into the male pronuclei of rat one-cell embryos (eggs), which contribute to the relative inefficiency of the procedure (Charreau *et al.* 1996). Coupled with the high relative expense and space allocation for rats, many transgenic facilities have not extended their capabilities beyond mice. Our experimental requirements for a variety of transgenic models of neuronal function and behaviour demand the use of rats, and we have therefore devoted considerable effort to the establishment of a transgenic rat facility.

Our current rat production protocol is based on our previous experience (Zeng *et al.* 1994; Flavell *et al.* 1996). However, we have modified particular variables in response to the reproductive characteristics of our new colony of animals in order to optimize egg production and microinjection. Currently, 40-day-old Sprague–Dawley (CD-Charles River Ltd) females are superovulated with FSH (Folligon, 40 i.u., I.P. at 09.30 h on day −3) and hCG (Chorulon, 30 i.u., I.P. at 10.30 h on day −1), and mated overnight with stud males. Eggs harvested on day 0 are microinjected in M2 media, cultured overnight (in M16 at 37 °C and 5% CO_2), and two-cell embryos transferred into pseudopregnant recipient females anaesthetized with halothane (inhalation).

Although our protocol has been optimized over the years, we continue to experience occasional reductions in egg yield, and marked reductions of survival *in utero*, which occur more frequently than in the mouse, and contribute to the overall relative inefficiency of this procedure in the rat. Despite these unexplained variations we have successfully generated multiple lines of transgenic rats (e.g. Burke *et al.* 1997), and have obtained efficiencies as high as 1·2% (transgenic offspring as a percentage of injected and re-implanted embryos, where $n = 600$ embryos), an efficiency that compares favourably with values obtained in some mouse facilities. However, it is apparent that further optimization of this procedure could increase the overall efficiency of transgenic rat production.

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Measurement of small movements

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There are many situations in health and exercise science where the unintrusive measurement of small movements is required, for instance chest expansion and deflation in respiratory physiology and tibial translation in anterior cruciate ligament reconstruction surgery. A new sensor is being developed that is simultaneously light, flexible and sensitive, as well as being easy to use. It is based on a conductive rubber material whose resistance is nearly linearly proportional to its length. The sensor can be made in a variety of sizes down to a few millimetres in length, and can be placed on the skin or incorporated into a holder which in turn is attached to, or worn by, the subject.

We will demonstrate the sensor in use backed up by data showing accuracy and precision under a variety of conditions.

A fluid-switch detector of air movement

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We have developed a simple device that is capable of detecting air movements of very low linear velocity.

It works on the principle that a jet of gas, because of its low mass, is easily deflected away from a detector by movement of air at right angles to the stream; this property is known as a 'fluid switch' and the forces involved can be calculated (Giles, 1962). Deflection takes place over a very narrow range of air velocities and so the device acts as a switch or detector rather than a measuring instrument. The threshold of detection can be set by altering the distance between the jet and detector tubes and the pressure of the gas delivered to the jet.

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Publication 71.

Davies,I. & Davies,A.(1999)

"Pseudospike" a dedicated signal generator.

J.Physiol. Proc.Vol.515, 45p,

'Pseudospike' – a dedicated signal generator for testing single nerve fibre recording apparatus

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Other workers who record from 'single fibres' in peripheral nerves may have experienced our problem when, after moving apparatus, allowing it to be used by students, after a period of disuse, or for no apparent reason, difficulties are encountered in obtaining good 'singles'.

The question arises 'is this a loss of operator skill or is there a problem with the recording apparatus?'. To continue using biological material to validate the apparatus under these circumstances is unwarranted.

We have therefore constructed a tiny dedicated signal generator whose circuit is shown in Fig. 1 and which will be demonstrated. Its electrodes are draped over the recording electrodes and it provides 'pseudospikes' against which the apparatus is calibrated.

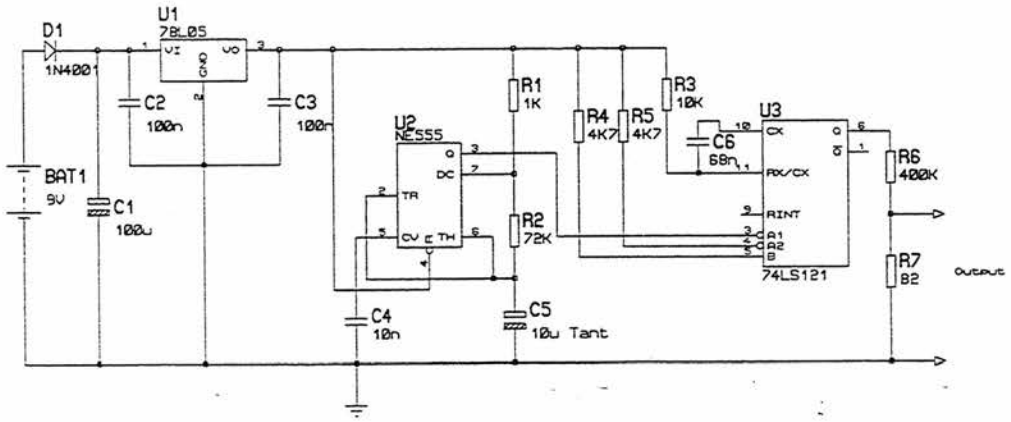

Fig. 1.

NEUREAL: real-time modelling software for integrating biological and simulated neurones and networks

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We have developed a user-friendly computer system that allows the transparent inclusion of biological neurones into real-time neural simulations. The software is designed to run under Windows 95 on Pentium class computers, and communicates at speeds of up to 100 kHz with biological neurones via conventional electrophysiological hardware. The heart of the system involves a user-defined database that takes advantage of the natural hierarchy that exists between neural modelling elements such as voltage- and transmitter-gated conductances, synapses, dendritic and axonal compartments, neurones and networks. All these elements can be easily created and combined to build realistic neurones and networks.

The system provides the possibility of performing simple dynamic clamp experiments and hybrid network construction. In addition, it enables the user to include biological neurones as single compartments in multi-compartment models: in this way, user-designed active dendritic tree structures can be attached to a biological neurone so that they become fully integrated electrical components. This provides an invaluable tool in the analysis of the effects of different distributions/properties of dendritic ion channels on neuronal behaviour.

Publication 72.

Davies,A.,Dallak,M. & Moores,C.(1999)

Profound morphological changes in an anaesthetised model of emphysema--.

J.Physiol,Proc.Vol.515, 92p.

Profound morphological changes in an anaesthetized rabbit model of emphysema produce limited changes in pattern of breathing

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It has long been accepted that the pattern of breathing of anaesthetized animals is modified by vagal afferent activity from receptors in the lungs, and that the activity of these receptors is modified by their physical and chemical environments. These environments change in disease.

For example, lung reflexes and receptor activity in a rat model of lung fibrosis are different from those in normal rats (Davies & Pack, 1991), and pulmonary receptor pattern of discharge is changed in a rat model of emphysema (Davies & Pirie, 1995). We would expect the structural changes seen in the lungs of models of emphysema to have a profound effect on pattern of breathing.

Pattern of breathing was measured by plethysmography in thirty-five Dutch Rabbits. Emphysema was then induced by a single intratracheal insufflation of porcine elastase 400 i.u. (kg body mass)$^{-1}$ under Hypnorm and Diazapam anaesthesia. After 4 weeks mean linear intercept in the model increased to $103{\cdot}2 \pm 6{\cdot}8$ μm compared with $75{\cdot}8 \pm 9{\cdot}6$ μm in a control group ($P < 0{\cdot}05$). Compliance of the excised lungs of the emphysematous rabbits was $6{\cdot}4 \pm 1{\cdot}2$ ml cmH_2O^{-1} and in the normals $4{\cdot}5 \pm 0{\cdot}49$ ml cmH_2O^{-1} ($P < 0{\cdot}01$) measured at the end of the acute experiments. Inspiratory time (t_I) and expiratory time (t_E) were shorter than before insufflation (t_I: $0{\cdot}53 \pm 0{\cdot}02$ s before, $0{\cdot}43 \pm 0{\cdot}11$ s after; t_E: $0{\cdot}66 \pm 0{\cdot}19$ s before, $0{\cdot}51 \pm 0{\cdot}12$ s after) but not statistically significantly so.

Anaesthetized with urethane (6·0 ml kg^{-1} 25% solution) and intubated, the diseased rabbits breathed with t_I of $0{\cdot}51 \pm 0{\cdot}02$ s and t_E of $0{\cdot}40 \pm 0{\cdot}01$ s, compared with the normal group: t_I $0{\cdot}38 \pm 0{\cdot}01$ s; t_E $0{\cdot}47 \pm 0{\cdot}01$ s.

Because both rapidly adapting pulmonary receptor (RAR) and slowly adapting pulmonary receptor (SAR) activity were found to change in our previous study of a rat model of emphysema (Davies & Pirie, 1995), we provoked lung inflation and deflation reflexes before and after highly specific blocking of SAR activity to separate the effects of these two groups (Davies *et al.* 1978).

The major reflex differences between the diseased and normal rabbits was after SAR block with a significantly shorter duration of t_E during eupnoea in the diseased rabbits ($0{\cdot}58 \pm 0{\cdot}02$ *vs.* $0{\cdot}50 \pm 0{\cdot}02$ s, $P < 0{\cdot}05$) and an augmentation of t_I in the diseased rabbits, during eupnoea and on lung deflation with a pressure of -10 cmH_2O ($0{\cdot}56 \pm 0{\cdot}02$ *vs.* $0{\cdot}64 \pm 0{\cdot}03$ s, $P < 0{\cdot}01$).

We are substantially in agreement with Mansoor *et al.* (1997) that profound emphysematous morphological changes in lung structure do not significantly alter vagally mediated pulmonary reflexes, but we maintain that there is a difference in reflex sensitivity that can be demonstrated by blocking SAR. (All values = means ± S.E.M.; P by the Mann–Whitney test.)

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Mustard oil evokes site-dependent alterations in hindlimb withdrawal reflexes in the decerebrated rabbit

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In the decerebrated, spinalized rabbit, noxious mechanical and chemical stimulation of the heel enhances reflexes evoked in medial gastrocnemius (MG) motoneurones evoked by electrical stimulation at the heel (Clarke *et al.* 1992), *and* responses of tibialis anterior (TA) motoneurones elicited by electrical stimulation at the toes (Clarke *et al.* 1991). When the same stimuli are applied to the toes, the heel-MG reflex is inhibited after mechanical but not chemical stimulation, whereas the toes-TA response is facilitated after both stimuli (Taylor *et al.* 1990; Clarke *et al.* 1991). The present study has examined the extent to which these stimulus-induced changes in withdrawal reflex pathways are preserved when the spinal cord is intact, a situation in which ascending–descending neuronal loops can influence events in the spinal cord.

Experiments were performed in six rabbits decerebrated to the precollicular level under halothane/nitrous oxide anaesthesia. Reflexes were evoked by constant current stimuli applied alternately to the heel and to the toes through percutaneous electrodes, and responses were recorded from the ipsilateral MG and TA muscle nerves to be averaged and integrated by computer. Mustard oil (Aldrich) was used as the conditioning stimulus. The depilated skin of both the heel and the toes were painted with mustard oil in each experiment. It was applied at one site for 3 min before washing with 0·9% saline. Reflexes were then recorded for at least 1 h before mustard oil was applied to the other site. The results are summarized in Table 1.

Publication 73.

Kelly,K.P,Moores,C.,Stenson,B.,Davies,A.&
Drumond,G.B.(1999)

The effect of perflurocarbon PF5080 on lung slowly adapting receptors.

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The effect of perfluorocarbon PF 5080 on lung slowly adapting receptors (SARs) in rabbits

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Partial liquid ventilation, a hybrid technique where conventional gas ventilation is conducted through a perfluorocarbon (PFC) medium is an experimental technique to support the injured lungs (Hirschl *et al.* 1995). The effects of the PFC on SARs has not been investigated. We investigated the effects of the PFC PF5080 (3M Industrial Chemical Products) on SAR discharge in rabbits.

Eight New Zealand White rabbits (3·52–4·54 kg) were anaesthetized with 25% urethane (6 ml kg^{-1}) intravenously. A tracheostomy was fashioned (3·0 mm i.d. endotracheal tube) and 0·2 mg kg^{-1} pancuronium was administered, followed by 0·15 mg kg^{-1} h^{-1}. 7 ml kg^{-1} h^{-1} of 0·45% saline/4% dextrose maintenance fluid were infused. Ventilation was maintained using an SLE 250 paediatric ventilator set to pressure-controlled mode (F_{I,O_2} 1·0, 4 cmH_2O PEEP, peak inspiratory pressures 13·6–20 cmH_2O, constant for each respective rabbit). Ventilation was monitored with a Ventrak pneumotachograph.

One vagus nerve was exposed in the neck. After desheathing, recordings were made using bipolar platinum electrodes from thin filaments containing one or two fibres arising from lung SARs identified by their discharge pattern during respiration. Nerve impulses were displayed on an oscilloscope, fed to a loudspeaker and converted into 5 V electronic pulses by a windows discriminator (Neurolog, Digitimer Ltd). Data were recorded onto videotape (TEAC XR 30). Patterns of discharge were analysed subsequently using spike counting software (Spike II for Windows 3.01, Cambridge Electronic Design).

Measurements were made under baseline conditions, after intratracheal administration of 5 ml of 0·9% saline, after 5 ml PF5080 and after filling the lungs to an approximate FRC of 20 ml kg^{-1} with PF5080. Euthanasia was with an overdose of anaesthetic.

Table 1. Maximum and minimum mean (95% confidence interval) discharge frequency (Hz) of SARs

	5 ml saline		5 ml PF5080		FRC PF5080	
	Pre	Post	Pre	Post	Pre	Post
Minimum	23	27	34	41	34	45
	(15)	(15)	(11)	(15)	(19)	(20)
Maximum	74	70	78	74	76	75
	(5·9)	(20)	(12)	(13)	(14)	(18)

Statistical analysis was with repeated measures ANOVA with Bonferroni's multiple *post hoc* comparisons test (GraphPad Prism 2.01, GraphPad Software Inc.).

There was no significant difference between Pre and Post intervention values for the addition of saline, 5 ml of PF5080 or filling to FRC with PF5080 ($P > 0·05$).

We were unable to demonstrate a change in receptor discharge frequency following the administration of PF5080 in this animal model.

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Hirschl, R.B., Pranikoff, T., Gauger, P., Schreiner, R.J., Dechert, R. & Bartlett, R.H. (1995). *Lancet* **346**, 1201–1202.

The effect of submucosal diathermy on unilateral nasal airflow in patients with rhinitis

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Studies on the nose often fail to take into account that the nose consists of two anatomically distinct nasal passages, each with a separate vasculature and a separate autonomic nerve supply. Studies investigating the effectiveness of surgical treatment of chronic nasal congestion have mainly used total resistance to airflow as a measure of nasal congestion. However, changes in total resistance are not related to changes in resistance in the separate nasal passages since unilateral resistance to airflow exhibits spontaneous changes over a number of hours that are sometimes referred to as a 'nasal cycle' (Eccles, 1982).

We propose that the minimum (F_{min}) and maximum (F_{max}) unilateral nasal airflow for each nasal passage provide a useful measure of the effects of nasal surgery on nasal congestion, since they give the range of nasal airflow in each nasal passage. F_{min} may be related to a phase of maximal vasodilatation and F_{max} related to a phase of maximal vasoconstricton of the nasal venous sinusoids (Flanagan & Eccles, 1998).

In this study we measured unilateral nasal resistance to airflow in twenty-seven patients with chronic rhinitis (age 13–57 years) with recordings made every hour over a 6 h period before and 2–3 months after submucosal diathermy of the inferior nasal turbinates. Measurements were obtained by the procedure of posterior rhinomanometry, with one nasal passage sealed with blenderm tape. Nasal airflow was recorded at a sample pressure of 75 Pa and the results are given as medians with interquartile range. The experimental protocol was approved by the local ethics committee.

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Chapter 22

The carriage of gases in the blood

A.S. Davies and C. Moores

Introduction

Nowhere in the human body is the concerted influence of multiple systems more evident than in the transport of gases by the blood. Pulmonary ventilation results in appropriate partial pressures of gas in the alveoli, and in turn in the formed and non-formed elements of blood. These are subsequently propelled through the tissues by the cardiovascular system. The unique properties of blood enhance the carriage of certain gases. The clinician may be concerned with the transport of many gaseous substances that include anaesthetic agents. This chapter, however, is primarily concerned with the physiologically important gases oxygen, O_2, and carbon dioxide, CO_2.

Oxygen is important because of the hundreds of metabolic processes into which it enters. By far the most quantitatively important is the adenosine triphosphate (ATP) producing cytochrome c oxidase system of mitochondria. This oxidative phosphorylation process accounts for 90% of our oxygen consumption and is the principal source of phosphate bond energy important in driving a wide range of metabolic reactions. The end-products of these reactions frequently include carbon dioxide. Carbon dioxide itself exerts profound physiological effects, particularly when present at elevated levels. These effects arise mainly from its acidic properties. There are thus many clinical situations when tissue levels of oxygen and/or carbon dioxide have to be controlled, usually by modifications to ventilation or to inhaled gas composition. These interventions depend on the efficiency of the blood in carrying such changes to the tissues. This chapter deals with the properties of blood which have evolved to facilitate gas transport. Some of the variables describing blood in terms of O_2 are given in Table 22.1.

Table 22.1 Oxygen transport – some values for normal blood

		Systemic arterial blood	*Mixed venous blood*
Tension	(mmHg)	100	40
	(kPa)	13.3	5.3
Blood content	(ml litre^{-1})	200	150
Saturation	(%)	98	75
Dissolved in plasma	(ml litre^{-1})	3	1.3
Haemoglobin	(gm litre^{-1})	150	150

Carriage of oxygen

Most of the oxygen transported in normal blood is carried in red cells. However, oxygen reaches the red cells in the lungs and subsequently moves to the tissues from the red cells, in simple solution in plasma and tissue fluid in which it is relatively poorly soluble. The amount of gas passively dissolved in a liquid is proportional to its partial pressure (Henry's Law). At a P_{O_2} of 13 kPa, 1 litre of plasma dissolves only 3 ml of O_2 in simple solution. An increase in the partial pressure of inhaled oxygen to 300 kPa (3 atmospheres of pure O_2) would theoretically supply the entire resting oxygen requirement through its carriage in simple solution. Alternatively, it would be possible adequately to supply our tissues with oxygen dissolved in plasma by increasing tissue perfusion by the circulation 50-fold. The circulatory system that has evolved in its present form provides an adequate tissue oxygenation through the properties of the respiratory pigment haemoglobin in the erythrocytes.

Synthetic fluids have been used as alternatives to whole blood as O_2 transporters. These usually consist of emulsions of high molecular weight fluorocarbons. The viscous properties of such emulsions are haemodynamically favourable for enhancing flow. However, the O_2 is carried in simple solution at concentrations linearly related to the inhaled partial pressure of O_2. The patient must therefore breathe substantially higher oxygen partial pressures, close to 100% O_2 at atmospheric pressure, if the resting requirement of 5 ml O_2/100 ml 'blood' is to be met.

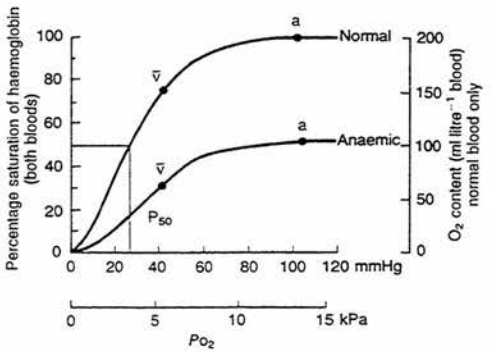

Figure 22.1 The oxyhaemoglobin dissociation curve.

Haemoglobin

The importance of haemoglobin (Hb) to life is probably only exceeded by that of chlorophyll which it closely resembles in molecular form (the Fe^{2+} of Hb being substituted by Mg^{2+} in chlorophyll). The Hb molecule consists of four subunits, each of which contains a prosthetic haem group and a polypeptide chain which makes up the globin part of the molecule. The haem groups are formed from a combination of protoporphyrin with ferrous iron. The 574 amino acids and the 4 haems give Hb an overall molecular weight of 64.4 kDa. Variations in the polypeptide chains can profoundly influence the O_2-carrying properties of the molecule. These variations give rise to the normal and abnormal forms of Hb (Bartels and Bauman, 1977). Because each molecule of Hb contains four O_2 binding sites the conventional reaction describing the formation of oxyhaemoglobin, HbO_2:

$$Hb + O_2 = HbO_2$$

should really be regarded as part of a four-step sequence that culminates in the overall reaction:

$$Hb_4 + 4O_2 = Hb_4\,O_8.$$

Each reaction step represents the attachment of a single O_2 molecule to the iron atom of a single haem group. Each attachment distorts the Hb molecule and changes its affinity for the next O_2. This allosteric effect explains the sigmoid shape of a plot of haemoglobin saturation against P_{O_2} (Fig. 22.1). Since the Fe^{2+} does not change its oxidation state, this reaction sequence does not involve chemical oxidation and reduction. It is nevertheless driven to the right in the above four steps by increased P_{O_2}.

Normal human blood contains around 150 g of Hb per litre. One gram of Hb can carry about 2 mg or 1.36 ml of O_2 at body temperature. Therefore normal blood can carry up to 200 ml of O_2 per litre at normal body temperature and pressure. The amount of oxygen that can be carried in a litre of blood is determined by

1. The *partial pressure* (units: kPa) of oxygen which determines the loading and unloading of O_2. Thus, venous blood, with an oxygen partial pressure, P_{O_2}, of 5.3 kPa is subject to a P_{O_2} of 13 kPa and therefore a *loading* pressure of 7.7 kPa in the lungs. In contrast, arterial blood, fully saturated to a P_{O_2} of 13 kPa is subjected to an *unloading* pressure of 12 kPa when it reaches active muscle tissue which has a P_{O_2} of 1 kPa.
2. The *haemoglobin content* (units: g l^{-1}). Thus, in anaemia, the Hb content of blood and therefore its potential to carry O_2 is reduced, irrespective of the saturation of Hb.
3. *The oxygen saturation* of Hb present (defined as the ratio of oxygen content to oxygen capacity. Both have the same units mass/volume and thus saturation has no units as it is a ratio. It is thus conventionally expressed as a percentage (%) rather than as a fraction). This quantity depends on the ambient P_{O_2} and is independent of the Hb concentration. For example, both the normal and anaemic blood represented in Fig. 22.1 are equally saturated at all values of P_{O_2}. As HbO_2 has a different absorption spectrum from Hb it is technically easy to measure saturation using spectrometry. A saturation of 100% implies blood is 'fully loaded' with O_2. However, it gives little information about important variables such as the amount and availability of that O_2. *Cyanosis* appears when capillary blood in the skin contains about 50 g l^{-1} of deoxyhaemoglobin. In normal blood this occurs at a saturation of 70% and therefore with a P_{O_2} of 5 kPa. In anaemia, because of the smaller amounts of deoxyhaemoglobin present, patients can be hypoxic without being cyanotic.

Oxygen loading and unloading

The oxyhaemoglobin dissociation curve

Both the position of the oxyhaemoglobin dissociation curve and its shape are of physiological importance (Bauman *et al.*, 1987). The shape can be described in terms of so-called loading and unloading regions.

The loading region

This is the flat region observed above oxygen partial pressures of about 10 kPa. The very small slope of this region reflects the full loading of blood with O_2, with each Hb molecule saturated by four O_2 molecules. In this event, any increase in P_{O_2} by increased ventilation or addition of O_2 to the inspired air would have no influence on the O_2 content of the blood that leaves the lungs. Conversely, a 25% decrease in normal alveolar ventilation would alter the partial pressure of O_2 in the alveoli but would not reduce the O_2 saturation of the blood. This characteristic of the oxyhaemoglobin curve reduces the effect of changes in ventilation produced by everyday activities upon arterial oxygen saturation.

The unloading region

The blood that flows through active tissues is subject to oxygen partial pressures associated with the steep part of the dissociation curve, at which small falls in P_{O_2} cause a large transfer of O_2. Such transfers of O_2 persist until the flat phase of the dissociation curve is reached. There, changes in P_{O_2} again do not produce large fluxes of O_2. The latter region of the curve is not normally reached under normal circum-

stances. In anaemic blood, however, there is a low content of O_2. Dissociation of oxygen then produces a rapid fall in Po_2 and oxygen saturation, and this may result in changes that extend to the lower horizontal portion of the curve.

Position of the oxyhaemoglobin dissociation curve

The position of the HbO_2 dissociation curve along the Po_2 axis is of paramount physiological importance. This can be considered in terms of a curve frequently simplified into three characteristics: the Po_2 at its origin, 100% saturation and at 50% saturation (or P50) resectively. The P50 for normal adult arterial blood is at about Po_2 of 3.2 kPa.

Displacement of the oxyhaemoglobin dissociation curve

The position of the HbO_2 curve is not fixed. With each circulation of the blood from lungs to tissues and back to lungs conditions in the blood change, and these changes displace the curve in directions that enhance overall delivery of O_2 to the tissues. A number of physiological factors change the position of the curve.

Carbon dioxide

This is released from tissues during cellular respiration, and results in an increase in extracellular $[H^+]$ through the carbonic anhydrase reaction. The increase in $[H^+]$ causes a positive displacement of the dissociation curve, that is, to the right. This facilitates the unloading of O_2. This useful mechanism does not persist in hypercapnia of several hours standing, with chronic acidosis. Under these chronic conditions, red cell levels of 2, 3-diphosphoglycerate are decreased and this returns the curve to a more normal position.

Hydrogen ions

Although CO_2 is the greatest acid load imposed on the body, any hydrogen ions produced by the tissues, or introduced by other means, will cause a shift of the HbO_2 dissociation curve to the right. This is termed the Bohr shift. A decrease in physiological pH of 0.2 units can increase O_2 release by 25% and hence enhances the supply of O_2 to metabolizing tissue.

Temperature

Reduction in temperature moves the HbO_2 curve to the left. This effectively inhibits O_2 release. In patients made hypothermic for, e.g. cardiac surgery; blood leaving the tissues may therefore be relatively saturated in the face of low tissue Po_2.

2, 3-Diphosphoglycerate

In red blood cells, in addition to the mechanism which converts 1, 3-diphosphoglycerate to 3-phosphoglycerate with the production of adenosine triphosphate in the glycolytic pathway, 'shunt' reactions convert 1, 3-diphosphoglycerate to 2, 3-diphosphoglycerate (2, 3-DPG). The product 2, 3-DPG is important for the function of red cells as it promotes the release of O_2 under certain long term conditions. These include:

i. *Chronic hypoxia.* This causes an increase in red cell levels of DPG and consequently a more ready release of O_2 in hypoxic tissues.
ii. *Prolonged exercise.* Also increases erythrocyte 2, 3-DPG, by producing hypoxia, or by some other mechanism.
iii. *Storage* of blood decreases its 2, 3-DPG content and reduces its tendency to release O_2. Such blood may be treated to restore its 2, 3-DPG content and so reverse this condition.
iv. *Abnormal haemoglobins* are frequently associated with abnormal levels of 2, 3-DPG.
v. *Enzyme abnormalities* of the red cells such as pyruvate kinase or hexokinase deficiencies, respectively, increase and decrease red-cell 2, 3-DPG content.

The shifts in the dissociation curve produced by the physiological mechanisms outlined above are of considerable clinical importance because tissue Po_2 is closer to the venous point, on the steep section of the curve, than the arterial point, on the flat section. Thus, shifting the curve to the right will raise venous and tissue Po_2. In respiratory acidosis this effect is exaggerated, producing a vigorous unloading of O_2 at the tissues.

Myoglobin and oxygen stores

It is an important fact that the reserve of oxygen in the body is perilously small. Small reserves mean that changes in the *supply* of O_2 produced by changes in ventilation or in the Po_2 of the inspired air rapidly manifest as changes in the Po_2 of the tissues. For example, half of a stepwise change in O_2 supply would be reflected in arterial blood within 30 s. The composition of gas contained in the lungs offers a means of greatly augmenting O_2 stores. Breathing pure O_2 triples the O_2 stores, as demonstrated by the ability under these conditions, to breath-hold for almost 10 minutes without becoming hypoxic.

Myoglobin

Myoglobin occurs in skeletal and cardiac muscle. It provides a small but significant O_2 store. The oxymyoglobin dissociation curve for myoglobin lies well to the left of the HbO_2 dissociation curve and so only releases O_2 at much lower Po_2. Oxymyoglobin probably provides a small O_2 store, depleted in seconds, which releases oxygen under the anaerobic conditions found occasionally in skeletal muscle and regularly in cardiac muscle.

Fetal haemoglobin

Fetal haemoglobin has a dissociation curve similar to that of myoglobin. This facilitates the transfer of O_2 from mother to fetus. Because of the relative positions of the curves, fetal blood is far more saturated than maternal blood at most physiological Po_2 values.

The double Bohr shift

This also aids transfer of O_2 from mother to fetus. In this phenomenon, CO_2 formed by the fetus first displaces the fetal oxyhaemoglobin curve to release O_2. The CO_2 then

diffuses across the placenta into maternal blood. There it repeats the process of O_2 displacement in maternal oxyhaemoglobin and thereby frees more O_2 for the fetus. However, the high affinity of fetal haemoglobin for O_2 means that the fetal tissues are generally on the verge of hypoxia, which fetal tissues seem better able to resist than adult tissues. Fetal haemoglobin disappears from the blood in a few months after birth.

Variant forms of haemoglobin

As well as these 'normal' forms of human haemoglobin, many other forms exist. Most animal species have their own forms. In man well over a hundred variants of the four polypeptide chains that make up the molecule have been identified. Some have abnormal dissociation curves, either because of properties of the Hb itself or because of their reaction to other red cell components such as the 2, 3-DPG content. For example, the physical properties of Hb can be changed by the seemingly trivial but specifically sited substitution of valine for glutamic acid in the globin chain. This causes a critical loss of Hb solubility and results in the hereditary condition of sickle cell anaemia. A change of the valency of the iron of Hb to the ferric form produces *methaemoglobin* which cannot combine with O_2. Poisoning by higher oxides of nitrogen is largely due to the formation of methaemoglobin. The process is slowly reversed by enzymes which are absent in familial methaemoglobinaemia. Such patients are often diagnosed as cyanotic because of the brownish-grey colour of methaemoglobin. Patients poisoned by carbon monoxide on the other hand present with a deceptively pink colour owing to the formation of cherry-red carboxyhaemoglobin. The affinity of carbon monoxide for Hb is more than 200 times greater than that for O_2. Thus 0.1% CO in the inspired air will eventually bind to 50% of the available Hb. Although carbon monoxide poisoning from car exhaust fumes represents the numerically greatest intentional or unintentional threat to health from this substance, levels of carboxyhaemoglobin of up to 10% have been found in tobacco smokers.

Blood O_2 tension

Important values that are related to oxygen transport in the blood are given in Table 22.1. A determination of arterial gas tensions allows us to assess how well the two fundamental processes of respiration-ventilation and gas exchange are being carried out, and to arrive at the diagnosis of *respiratory failure*. It should be remembered, however, that normality encompasses a wide range of values affected by a variety of factors, including age. It has been suggested that the mean arterial Po_2 for subjects at rest, at sea level and breathing air can be given by the relationship

$$P_{a_{O_2}} = 13.6 - 0.044 \times (\text{age in years}) \text{ kPa}.$$

Nevertheless, the 95% confidence limits (defining ± 2 standard deviations from the mean) extend ± 1.3 kPa above and below that value (Fig. 22.2). Thus while respiratory failure is sometimes defined as a value of $P_{a_{O_2}}$ that falls below 8 kPa, this would not be the case for a 70-year-old patient. The altered physiology of respiratory failure always produces a reduced $P_{a_{O_2}}$. Whether this is accompanied by a normal or a raised $P_{a_{CO_2}}$ has important implications for diagnosis and treatment.

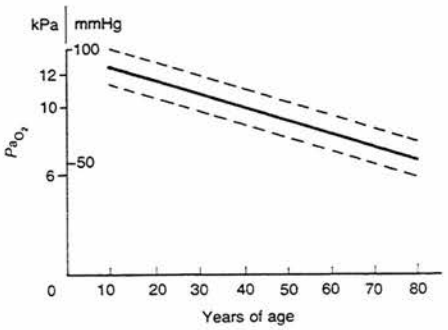

Figure 22.2 Change of arterial Po_2 with age. (Values for 95% of the population lie between the dotted lines.)

Measurement of blood oxygen

The partial pressure of O_2 in arterial blood is the cardinal index of the efficiency of respiration. Partial pressure and other variables relating to blood oxygenation can be measured *directly* on an arterial sample taken without exposure to air, and with consideration for its continuing metabolism and changes in temperature, or, *indirectly*, by measurements made on the surface of the skin. Indirect methods of measuring saturation by spectrophotometry and Po_2 measured polarographically are now sufficiently reliable for use in clinical monitoring.

CO_2 transport and blood buffering

The word oxygen means acid producer. Our oxidative metabolism produces a considerable daily acid load which must be dealt with. The lungs are quantitatively by far the most important organ of acid excretion. In a normal day an adult's kidneys excrete 50–90 milli-equivalents (mEq) of acid (i.e. millimoles of H^+). The lungs excrete about 13 000 mEq in the form of carbon dioxide (Bauer *et al.*, 1980). This CO_2 formed at the tissues is transported to the lungs in the blood. Ten times more CO_2 than O_2 by volume is carried in simple solution in the blood. But like O_2, most of the CO_2 is carried by more complex physicochemical means subserved by highly evolved physiological mechanisms. Normal values for variables relating to CO_2 transport by the blood and blood buffering are given in Table 22.2.

Carbon dioxide is added to capillary blood by diffusion from its sites of production within metabolizing cells. Diffusion of CO_2 through an aqueous medium is about 20 times as rapid as is that of O_2. The total flux of CO_2 into or out of the blood is, however, limited to approximately that of O_2 in the opposite direction by the smaller partial pressure difference that drives CO_2 diffusion, and the relatively slow speed of the chemical reactions involved.

The first step in the transport of CO_2 from the tissues by the blood is its dissolution in and partial reaction with plasma water

Table 22.2 Carbon dioxide transport–some values for normal blood

		Systemic arterial blood	*Mixed venous blood*
Tension	(mmHg)	40	46
	(kPa)	5.3	6.1
Total CO_2	(mM)	21	23
	(ml l^{-1})	480	520
In erythrocytes	*(/l blood)*		
Dissolved	(mmol)	0.4	0.5
Bicarbonate	(mmol)	5.8	5.9
Carbamino	(mmol)	1.1	1.7
In plasma			
(/l blood, Haematocrit 45)			
Dissolved	(mmol)	0.6	0.7
Bicarbonate	(mmol)	13.4	14.5
Acidity			
Plasma $[H^+]$	(nmol/l)	40	43
	pH	7.40	7.37

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \quad (22.1)$$

In plasma, this hydration of CO_2 takes almost 2 minutes to reach 95% equilibrium at body temperature (within the erythrocytes the reaction is catalysed: see below). The H^+ produced by the dissociation of carbonic acid so formed is buffered by plasma proteins.

Carbon dioxide also reacts with amino groups on both plasma proteins and haemoglobin to form carbamino compounds.

$$R\text{-}NH_2 + CO_2 \rightleftharpoons R\text{-}NH\ COO^- + H^+ \quad (22.2)$$

The majority of CO_2 carried this way is in association with haemoglobin which is present at four times higher concentrations than, and has three times the affinity for CO_2 of plasma proteins. Although the amount carried as carbamino compounds is small (Table 22.2) the difference between the amounts in arterial and venous blood contributes about one-third to the total CO_2 exchange as the blood passes through lungs or tissues. This makes up a large part of the *Haldane effect*, which is the effect on CO_2 carrying capacity of blood of oxygenation and deoxygenation.

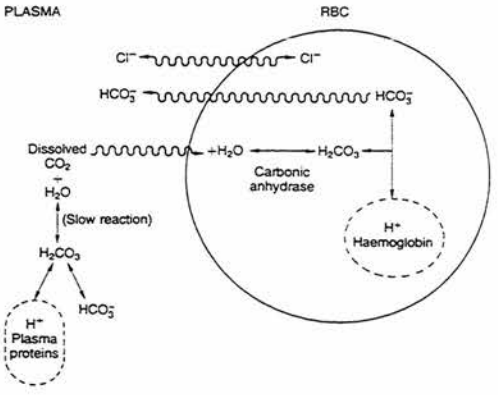

Figure 22.3 The transport of carbon dioxide as plasma bicarbonate.

Forms of transport

Table 2.22 describes how approximately 70% of CO_2 transported by blood is carried in the plasma (see also Klocke, 1987). However, the *exchange* of CO_2 between tissues or lungs and its major form of transport (plasma bicarbonate) depends almost exclusively on mechanisms within the red blood cells. The process is shown in Fig. 22.3. Carbonic anhydrase within erythrocytes catalyses the equilibrium between H_2O, CO_2 and H_2CO_3. Haemoglobin, particularly in its deoxygenated form, sequesters hydrogen ions that result from the dissociation of carbonic acid. The important role of carbonic anhydrase in promoting the loading and unloading of CO_2 at tissues and lungs can be demonstrated by pharmacological inhibition of the enzyme. Under these circumstances CO_2 in solution in the plasma builds up, creating acidosis.

The carbon dioxide dissociation curve

The relationship between partial pressure and content of CO_2 in whole blood can be plotted as for oxygen (Fig. 22.4). However, unlike the dissociation relationship for oxygen, that for CO_2 is almost linear over the physiological range and cannot be saturated. Furthermore, the 'true' physiological curve is steeper than that obtained for either oxygenated or deoxygenated blood because of the Haldane shift. There have been a number of diagrams published which relate the

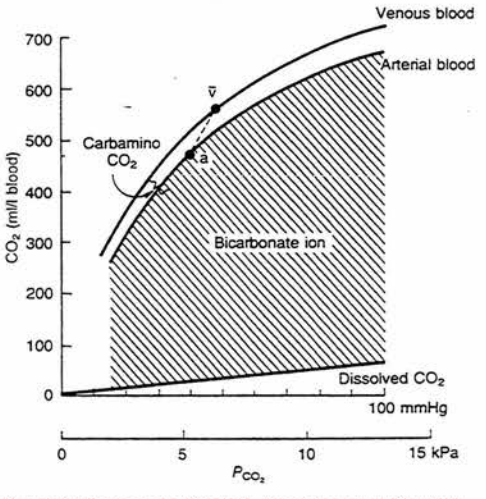

Figure 22.4 Components of the CO_2 dissociation curve for whole blood. The *in vivo* dissociation curve is the broken line joining the arterial (a) and mixed venous ($\bar{v}$) points.

plasma bicarbonate concentration, pH and P_{CO_2} respectively. Because these variables are absolutely related (by the Henderson–Hasselbalch equation, see below) any one variable can be derived from the other two.

Oxygen and carbon dioxide interactions

The loading and unloading of blood with O_2 and CO_2 at the lungs or tissues is a synergistic process with the movement of one gas facilitating the movement of the other through the Bohr and Haldane shifts (Perutz, 1990). The amounts of gases moved are not the same and this results in a reduction of about 7 kPa (50 mmHg) in the combined partial pressure of O_2 and CO_2 in blood as it passes through the tissues. Thus, a bubble of air in the tissues will lose O_2 to and pick up some CO_2 from the capillary blood. Since end-capillary blood always has a total partial pressure less than atmospheric pressure, O_2, CO_2 and N_2 are absorbed from the bubble into the blood. This process of absorption can be accelerated by giving the subject pure O_2 to breathe. Displacement of N_2 by O_2, which is metabolized, results in an even lower end-capillary combined partial pressure.

Acid–base balance in blood in vitro

The normal range of plasma pH is 7.35–7.45. Failure of physiological mechanisms to maintain the pH between these limits results in acidaemia or alkalaemia. This section deals with acid–base balance in the blood; however, the other two body fluid compartments (interstitial and intracellular fluids) contain much greater quantities of CO_2, H^+ and HCO_3^-. It is therefore important also to consider the interactions of the three compartments in clinical situations (Jones, 1980).

Buffering systems

Chemical systems which resist changes of pH by taking up or releasing H^+ are called *buffers*. Buffering properties usually result from the presence of organic or inorganic salts of weak acids. The most quantitatively important *chemical* buffers in blood are haemoglobin and plasma proteins. This action of taking up H^+ is shown in equation 22.2 where the functional group R– can represent either haemoglobin or plasma protein. Buffering systems can also result from the presence of radicals of inorganic acids. One such system in blood is phosphate, in its acidic ($H_2PO_4^-$) and basic (HPO_4^{2-}) forms. These buffer systems occur at concentrations too low to be of much importance in the blood. However, they may be more important in the intracellular space. They are particularly important in regulating acid excretion by the kidneys.

An important descriptive term for the properties of a buffer system is its pK_a. This is the negative logarithm of the dissociation constant of H^+ from the acid HA:

$$HA = H^+ + A^-$$

$$K_a = [H^+][A^-]/[HA]$$

$$pK_a = -\log_{10} K_a$$

The pK_a of a buffer system is the pH at which the buffer and acid are each half-dissociated.

$$\text{Thus } pK_a = pH + \log_{10}\left(\frac{[HA]}{[A^-]}\right)$$

and so, if $[HA] = [A^-]$

then $pK_a = pH$:

At a pH equal to the pK_a, the buffer system has the maximum 'room to move' in either direction in taking up or releasing H^+. It also shows the minimum of pH change in response to addition of acid or base. At its pK_a the buffer is therefore at its most effective at resisting changes in pH. It seems remarkable then that the pK_a of the *functionally* most important buffer in blood, the bicarbonate–carbonic acid system, is somewhat removed from the pH of plasma.

The bicarbonate–carbonic acid system

The buffering mechanism of this system can be represented by the equation

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The addition or removal of H^+ will shift the reaction to the left or right but changes in pH will be minimized. The Law of Mass Action gives:

$$\frac{[H^+][HCO_3^-]}{[H_2CO_3]} = K_a$$

where K_a is the dissociation constant of H_2CO_3.

The *Henderson–Hasselbalch equation* expresses this relationship in a more useful form relevant to CO_2 carriage of the blood. Taking the logarithm to base 10 of both sides of the equation and transposing gives

$$\log\left(\frac{[H^+][HCO_3^-]}{[H_2CO_3]}\right) = \log K_a$$

$$\log [H^+] + \log\left(\frac{[HCO_3^-]}{[H_2CO_3]}\right) = \log K_a$$

but pH and pK_a are the negative logarithms of $[H^+]$ and K_a. Therefore:

$$pH = pK_a + \log\left(\frac{[HCO_3^-]}{[H_2CO_3]}\right) \qquad (22.3)$$

Because the H_2CO_3 concentration in blood is so small it is difficult to measure. However, the concentration of H_2CO_3 is related to the amount of CO_2 present by the reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

with the reaction displaced far to the left.

It is therefore possible to rewrite equation 22.3 as:

$$pH = pK' + \log\frac{[HCO_3^-]}{[CO_2]}$$

The concentration term, $[CO_2]$, is proportional to the partial pressure P_{CO_2}; this gives the more conventional expression of the Henderson–Hasselbalch equation:

$$pH = pK' + \log\frac{[HCO_3^-]}{aP_{CO_2}}$$

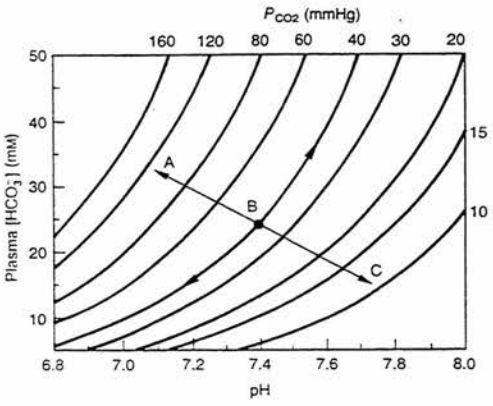

Figure 22.5 The Davenport pH/[HCO_3^-] diagram. The line ABC is the 'normal' dissociation curve for plasma CO_2 as described by the Henderson–Haselbalch equation. The line is straight, representing the line a-$\bar{v}$ in Fig. 22.4. Uncompensated respiratory acidosis (beyond A) or alkalosis (beyond C) moves the point B, representing normality, along this line. Uncompensated metabolic acidosis or alkalosis moves B along the P_{CO_2} isobar.

where a is the solubility coefficient of CO_2 at 38°C.

Because the numerator and denominator of this equation are governed by the kidneys and the lungs respectively both systems are important in regulating the HCO_3^-:CO_2 ratio and therefore the plasma pH. Thus, it has been suggested (Gilman and Brazeau, 1953) that the Henderson–Hasselbalch equation can be qualitatively written:

$$\text{pH} = \text{constant} + \frac{\text{kidney function}}{\text{lung function}}$$

This concept explains why the bicarbonate–carbonic acid system is so important as a physiological buffer although it is not a very powerful chemical buffer. It involves systems, the lungs and kidneys, that can directly regulate the quantities of the components of the buffer system (i.e. CO_2 and HCO_3^-), using metabolic energy, as opposed to other physiological buffer systems such as proteins which are metabolically expensive to replenish.

Acid–base balance calculations

The major reason for a quantitative assessment of acid–base status is the detection and quantification of pH abnormalities and their attribution to a respiratory or metabolic source. If any two of the variables (P_{CO_2}, pH or [HCO_3^-]) of the Henderson–Hasselbalch equation are known the third can be

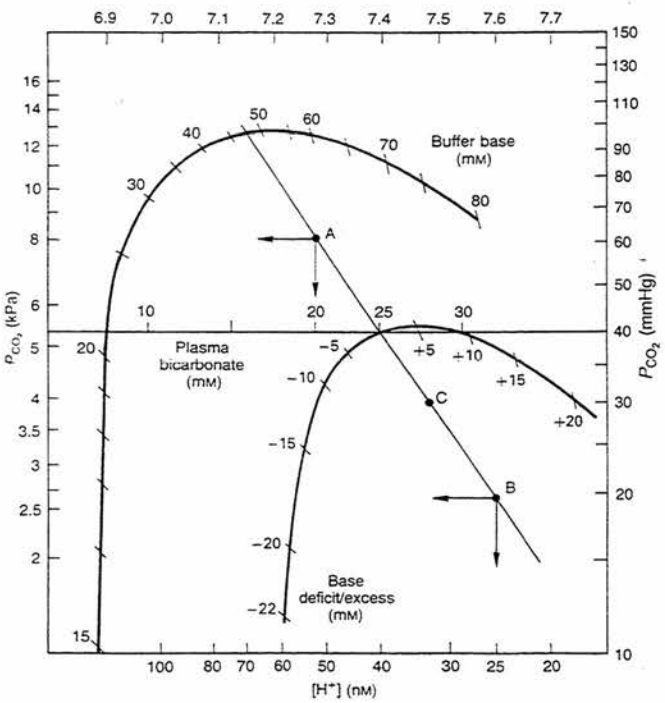

Figure 22.6 The Siggard-Anderson nomogram. When used to estimate P_{CO_2} and base deficit or excess of a patient's blood a sample is equilibrated with known P_{CO_2} gas mixtures A and B and pH measured. The pH of freshly taken blood then enables point C to be interpolated and its P_{CO_2} (30 mmHg) and base deficit (zero) read.

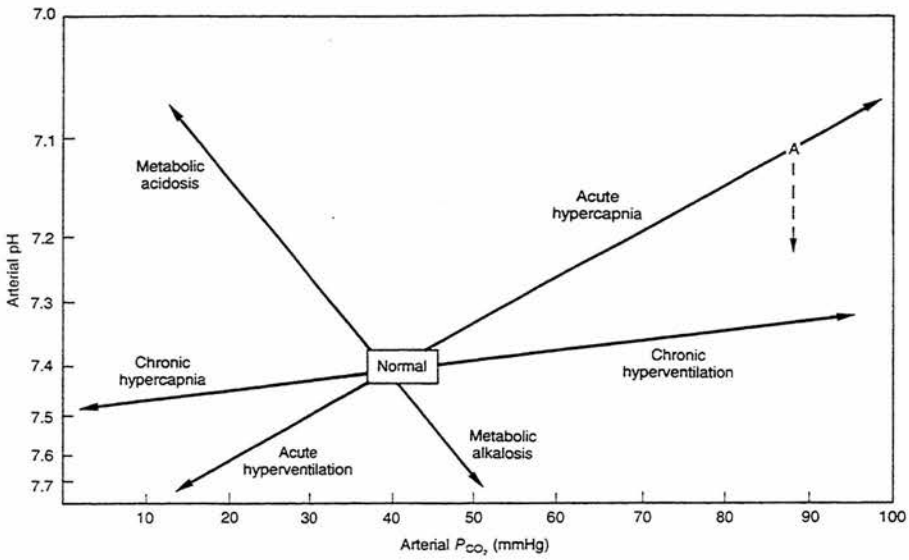

Figure 22.7 Changes in arterial pH and Pco_2 with different acid-base disturbances.

calculated (Fig. 22.5). In this way the acid–base status of the blood can be assessed.

$$pH = pK_a + \log\left(\frac{[HCO_3^-]}{[CO_2]}\right)$$

Since normal arterial blood has a pH = 7.4 and $pK_a = 6.1$ the normal ratio of CO_2 to HCO_3^- is given by:

$$7.4 - 6.1 = \log\left(\frac{[HCO_3^-]}{[CO_2]}\right) = \log\left(\frac{20}{1}\right)$$

Arterial blood can be drawn from the body, equilibrated with known Pco_2 and measurements of pH and $[HCO_3^-]$ then taken. It has been suggested that to be of any real value in assessing acid-base status these measurements should be made directly on arterial blood withdrawn after the *subject* had been equilibrated with known Pco_2 as only such measurements will describe what is happening in the body. In practice *in vitro* measurements prove adequate for most clinical situations. Two important variables which describe the acid–base status of blood are the *standard bicarbonate* and the *base excess, or deficit.*

Standard bicarbonate

This is the bicarbonate concentration (mM) that is measured in whole blood subject to a Pco_2 of 5.3 kPa (40 mmHg) and a temperature of 37°C. The purpose of fixing the Pco_2 in this measurement is to remove any *respiratory* perturbation of bicarbonate concentration in the blood. In practice this applied Pco_2 of 5.3 kPa is 'bracketed' by determinations made with the imposition of known higher and lower levels of Pco_2. The pH is measured at each of these partial pressures. A blood–buffer line is then drawn between the two sets of co-ordinates on a nomogram that relates pH, Pco_2 and $[HCO_3^-]$ according to the Henderson–Hasselbalch equation (Fig. 22.6) and the pH at 5.3 kPa taken. These calculations are now carried out automatically by most blood-gas analysers. If the bicarbonate concentration at a Pco_2 of 5.3 kPa is standard (25 mM) then any acid–base disturbance must have been exclusively respiratory. If there is not a normal standard bicarbonate at this Pco_2 then there is a metabolic component that can be quantified by measuring base deficit or excess.

Base deficit or excess

Fixing Pco_2 at 5.3 kPa, as described above will remove any respiratory component of acid–base imbalance. Measurement of pH at this stage will not, however, reveal how much H^+ has been added or removed from the blood by the buffering mechanisms in an attempt to minimize changes in pH. In other words measurement of the pH at a Pco_2 of 5.3 kPa will not show what deficit, or excess, of base has been entailed in attempting to maintain pH. To quantify this, the blood must be titrated back to a pH of 7.4 using HCl or NaOH, and the deficit or excess of base present thus calculated. In practice, these titrations are not usually carried out manually: modern blood gas anaysers provide this service. At a more fundamental and graphical level nomograms relating pH, Pco_2 and

$[HCO_3^-]$ can be used. Perhaps the two most popular are Davenport's pH/bicarbonate diagram (Fig. 22.5) and Siggaard-Andersen's pH/P_{CO_2} diagram (Fig. 22.6).

The positions occupied on such a nomogram by arterial blood exhibiting some common acid-base disturbances are shown in Fig. 22.7. It should be remembered that such conditions are not necessarily static. For example, the values represented by 'A' (Acute hypercapnia, perhaps due to hypoventilation) will gradually move toward the chronic line as the kidneys retain bicarbonate to compensate.

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Chapter 23

Pulmonary blood flow and the exchange of gases

A.S. Davies and C. Moores

The supply of oxygen to and the elimination of carbon dioxide from the body are essential for the maintenance of animal life. The carriage of these two substances to and from the lungs in the blood is discussed in detail in Chapter 22, and Chapter 24 covers the process of ventilation, which determines the composition of gas within the alveoli. This chapter is concerned with how oxygen and carbon dioxide pass between blood and alveoli, the functioning of the pulmonary circulation and the important influence that this has on the oxygenation of arterial blood.

Diffusion

The exchange of oxygen and carbon dioxide between the plasma and the alveoli, across the alveolar membrane, takes place exclusively by diffusion. Diffusion of gases is a passive process whereby there is a net transfer of molecules of a given gas from an area of high partial pressure to an area of low partial pressure. This definition usually assumes that the *total* pressure at the two areas is equal, and thus excludes a mass transfer of gases such as occurs during inspiration and expiration.

The rate of diffusion of a gas through a membrane is proportional to the area across which diffusion takes place, the difference in partial pressures across the membrane, and the solubility of the gas in the membrane. It is inversely proportional to the thickness of the membrane and to the square root of the molecular weight of the gas (see also Chapter 1).

The total area of all the alveolar membranes is very large, about 80 m^2 and its thickness is probably about 500 nm (this includes the alveolar pneumocyte and the vascular endothelial cell). In the case of oxygen, diffusion into the erythrocytes has to take place, and although these cells are closely apposed to the capillary wall, this probably represents the greater part of the net movement of the oxygen molecules.

The *transfer* of oxygen and carbon dioxide between blood and alveoli is complicated by the fact that most of the oxygen in the blood is bound to haemoglobin, and most of the carbon dioxide is carried in the form of bicarbonate or carbamino compounds. Transfer of both gases involves both diffusion and chemical reactions. In the case of oxygen, the chemical reaction is the binding of oxygen to haemoglobin, and in the case of carbon dioxide, the reactions that are involved include the release of carbon dioxide from carbamino compounds and the dehydration of bicarbonate.

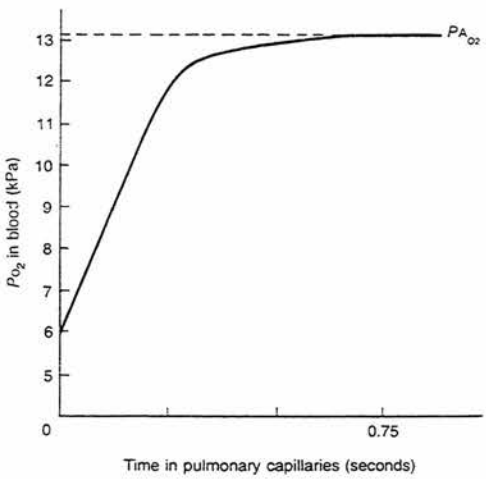

Figure 23.1 Change in partial pressure of oxygen in pulmonary blood during its passage through an alveolar capillary.

The binding of oxygen to haemoglobin is a complicated process (see Chapter 22) and, it seems, a relatively time consuming one. It is thought that the binding of haemoglobin takes slightly longer than its diffusion from the alveoli to the erythrocyte, making it the rate-limiting step in the transfer of oxygen.

Blood passes through an alveolar capillary in about 0.75 s and the changes in the partial pressure of oxygen which take place in this time are shown in Fig. 23.1. After about 0.25 s, or about one third of its transit time, the blood has an oxygen tension which is almost equal to that of alveolar gas. The transfer of oxygen is therefore sufficiently fast to ensure that the partial pressure of oxygen in blood leaving an alveolus is almost the same as the gas within it. This is true in normal subjects even during exercise when alveolar capillary transit times are reduced. It therefore follows that for a given alveolar partial pressure, the amount of oxygen that can be carried from the lungs is limited by the blood flow through the pulmonary circulation rather than the diffusion of oxygen.

The pulmonary circulation

The pulmonary circulation accounts for only 10–25% of the total blood volume, yet nearly the entire cardiac output passes through it. The flow of blood through the pulmonary and systemic circulations is therefore about equal, but the pressures in the pulmonary circulation are much less than those in the systemic circulation. The distribution of blood flow in the lungs is under local control and is regulated towards matching ventilation and perfusion within regions of the lungs, but is also influenced by other factors such as intra-alveolar pressure and gravity. The systemic and pulmonary circulations, although superficially similar, are different in a number of important respects, suiting their different functions (see also Chapter 21).

Functional anatomy

The pulmonary vascular system

The structure of the pulmonary and bronchial circulations provides important insights into their function. The pulmonary arterial trunk arises from the right ventricle and divides into the left and right pulmonary arteries which carry deoxygenated blood to the lungs. The pulmonary arteries divide and redivide, and pass through the lung tissue in association with airways until the level of the terminal bronchioles. At this point, a meshwork of capillaries is formed around the alveoli and here gaseous exchange takes place. To allow the maximum surface area between blood and gas, the network of alveolar capillaries is a particularly fine one, and it is helpful to think of the alveolar circulation as consisting of a flowing film of blood, interrupted by segments of vessel wall. Oxygenated blood passes from the alveolar capillaries into the pulmonary venules lying in septa between the lung lobules and thence by way of the pulmonary veins to the left atrium, there re-entering the systemic circulation.

The histological appearance of the pulmonary blood vessels reflects the fact that they carry blood at a much lower pressure than their systemic counterparts. The pulmonary arteries have a tunica media which is about half as thick as that of systemic vessels of a similar size and the arterioles of the pulmonary circulation possess very thin media containing virtually no muscular fibres. This reflects the fact that, unlike systemic arterioles, they offer very little resistance to blood flow, and play a very minor role in the regulation of pulmonary capillary blood flow. Pulmonary veins are very similar histologically to pulmonary arterioles and in a histological specimen, it is often difficult to tell them apart.

Anatomical shunting vessels

The above anatomical scheme applies to that part of the blood flow through the lungs which takes part in gaseous exchange, but the pulmonary circulation is, in fact, a little more complicated and the situation is depicted more fully in Fig. 23.2. Other components of the circulation associated with the lungs are described below. Some of these result in venous blood being added to oxygenated blood. This is termed *shunting* and results in a decrease in the oxygen content of the arterial blood.

The *bronchial circulation* consists of vessels that arise ultimately from the aorta. These supply oxygenated blood to lung and bronchial tissue but this blood takes no part in alveolar gas exchange. Blood in the bronchial circulation returns to the heart in two ways. The pleurohilar veins arise from the hila of the lungs and drain into the azygos veins.

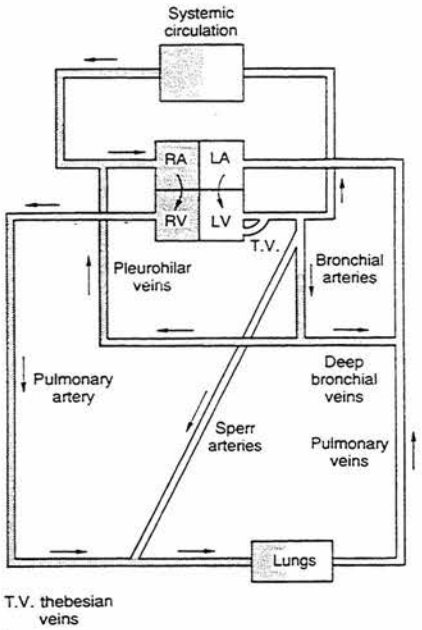

Figure 23.2 The bronchial circulation. Deoxygenated blood shown hatched.

This part of the bronchial circulation may therefore be considered as part of the systemic circulation. The deep bronchial veins, however, drain into the pulmonary veins, thereby forming a shunt. The bronchial circulation is probably not of very great importance in normal subjects. After lung transplantation, the bronchial circulation is not re-established, with apparently few ill effects.

The left-sided *Thebesian veins* provide another source of deoxygenated blood entering the systemic circulation. These veins arise in the myocardium and drain blood from the coronary circulation directly into the left ventricle.

The *'sperr arteries'* are connections between the bronchial arteries and the venous side of the pulmonary circulation. They are therefore capable of carrying oxygenated blood from the systemic to the pulmonary circulation. In normal subjects, it is thought that the blood flow through these vessels is very small, although they may play a more significant role in diseases such as pulmonary hypertension.

Congenital heart disease which results in a significant right to left shunt can be an important cause of deoxygenated blood entering the left hand side of the circulation.

Pressures within the pulmonary circulation

Pressures within the pulmonary circulation are much lower than those found in the systemic circulation, and are shown in Fig. 23.3. The pressure within the pulmonary artery is approximately 28/8 mmHg, compared with 120/80 mmHg in the aorta, whereas the left and right atrial pressures are roughly equal at about 2–3 mmHg. The pulse pressure in the pulmonary artery is therefore about 16 mmHg, about two thirds of the value of the systolic pressure. As a result, the flow of blood through the pulmonary circulation is much more pulsatile than that through the systemic circulation where the pulse pressure is only one third of the systolic pressure. Indeed, the blood flow through the pulmonary capillaries is also pulsatile, in contrast to the steady flow through the systemic capillaries.

In the systemic circulation, most of the drop in blood pressure between the arterial and venous sides takes place at the end of the high resistance arterioles immediately before the capillaries. The pressure in these capillaries is about 30 mmHg, a value which is much closer to right atrial pressure than the aortic pressure. In contrast, the capillary pressure is thought to be approximately 7 mmHg in the pulmonary circulation. This value lies about half way between the mean pulmonary artery pressure of 13 mmHg and the left atrial pressure. This suggests that the resistances of the arterial and venous sides of the pulmonary circulation are probably about equal and that the pulmonary arterioles are likely to play only a small role in the regulation of capillary blood flow.

When considering the systemic circulation it is customary to measure the intravascular pressure with reference to the atmosphere. In the pulmonary circulation, however, it is often more useful to consider the *transmural pressure*. This is the difference between the pressure within and the pressure immediately outside the vessel. The pressure outside the larger pulmonary vessels is approximately equal to the intrapleural pressure, whereas the pressure immediately outside the pulmonary capillaries is about equal to the alveolar pressure.

Pulmonary capillaries are unique in that they are not supported to any great extent by connective tissue.

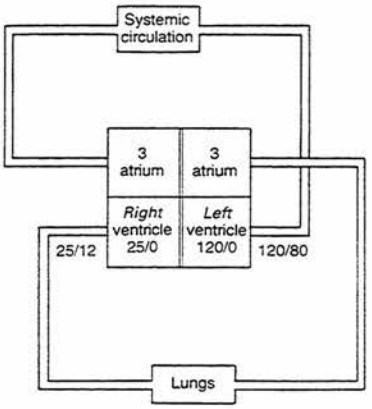

Figure 23.3 Pressures within the pulmonary and systemic circulation.

Consequently, should the alveolar pressure exceed the pressure within the vessels themselves (i.e. there is a negative transmural pressure) the vessel will tend to collapse. This is of importance in the distribution of blood flow within the lungs and is considered in more detail later. However, through most of the lungs, the capillary transmural pressure is positive. It would be large enough to cause the extravasation of fluid into the alveoli were it not for the presence of plasma proteins producing an oncotic pressure of about 30 mmHg opposing this effect. This balance is upset by an elevation of pulmonary blood pressure or by a reduction in plasma oncotic pressure, possibly leading to pulmonary oedema.

Measurement of pulmonary blood pressures

Pulmonary artery pressure is measured relatively easily using a pulmonary artery catheter. In its simplest form, this consists of a long catheter with a small balloon just proximal to its tip. This can be inflated through a port at its proximal end. The catheter is filled with fluid and inserted into the circulation via a central vein and negotiated through the right atrium and right ventricle and into the pulmonary artery. The course of the catheter tip through the right side of the heart is aided by the inflation of the balloon. Its position can be followed by monitoring the pressure of the fluid in the catheter at its proximal end as the right ventricle and pulmonary artery as each have a characteristic pressure waveform.

An estimate of the left atrial pressure can also be obtained using a pulmonary artery catheter. With the balloon inflated, the catheter is advanced down the pulmonary artery until the arterial pressure waveform becomes much flatter. This indicates that the balloon has wedged in and has occluded a large branch of the pulmonary artery. There now exists an

almost static column of blood between the tip of the catheter and the left atrium and measurement of the pressure in the catheter at this point yields the *pulmonary capillary wedge pressure (PCWP)* which in normal subjects is just slightly higher than the left atrial pressure. After measurement of the PCWP it is clearly advisable to deflate the balloon at the tip of the catheter to restore blood flow to all of the lung (see also Chapter 20).

Blood flow through the lungs

The total volume of blood in the pulmonary circulation is of the order of 500 ml of which about 80 ml occurs within the alveolar capillaries. The lungs receive nearly but not all of the cardiac output. Their total blood flow is therefore about 5 l min^{-1} at rest, increasing up to around 30 l min^{-1} during exercise. However, the distribution of this blood flow within the lungs is not even; it is influenced by a number of factors, including gravity, cardiac output, lung inflation and by regional differences in oxygen concentration, all of which are considered below.

Gravity

Gravity tends to direct the flow of blood towards the bases of the lungs. At the bases, the pressure at the right ventricle is augmented by the hydrostatic pressure of a column of blood extending from the pulmonary artery to the lung base. In contrast, at the apices, hydrostatic pressure reduces the pulmonary artery pressure such that the right ventricle is only just able to perfuse that region (see Fig. 23.4).

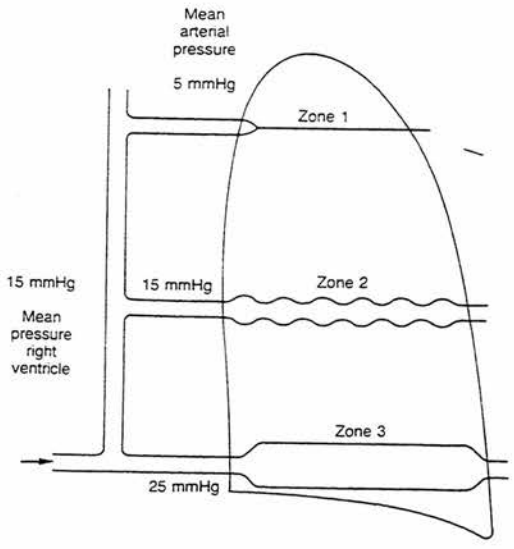

Figure 23.4 Arterial pressure at different heights within the upright lung.

Bearing in mind that the alveolar capillaries are liable to collapse when the pressure outside them exceeds that within them, some workers have described three zones within the lungs.

In *zone 1*, the alveolar pressure is greater than the pressure at the arterial end of the pulmonary capillaries. Under these circumstances the capillaries collapse and there is consequently no blood flow. Zone 1, if it occurs at all in healthy subjects, is confined to a small area at the apices of the lungs. In *zone 2*, the alveolar pressure is intermediate between the pressure at the arterial end of the capillary and that at the venous end. Under these circumstances, the blood flow is dependent upon the difference in pressure between the arterial end of the capillary and alveolar pressure, and is not influenced by the venous pressure. This zone probably occupies most of the upper and middle parts of the lungs. In the lower parts of the lungs is *zone 3*. Here, both the pressure at the arterial end of the capillaries, and that at the venous end exceed the alveolar pressure. In these capillaries flow is dependent upon the difference in hydrostatic pressure between their arterial and venous ends, and is not influenced by alveolar pressure.

Cardiac output

Increasing cardiac output causes a relatively modest increase in pulmonary artery pressure, suggesting that a simultaneous decrease in pulmonary vascular resistance takes place. It is thought that additional capillaries open to allow blood flow as vascular pressure increases. This increases the total capillary cross sectional area and decreases the resistance. Each capillary has its own critical opening pressure below which it is collapsed and above which it opens and carries blood. At higher pulmonary artery pressures, the opening pressure of a larger number of capillaries is exceeded. This phenomenon, known as *recruitment*, is probably most important in zones 1 and 2. Thus most of the additional blood flow at higher cardiac outputs is directed to these areas. Distension of capillaries which are already patent is also likely to play a role in this reduction of vascular resistance and is probably more important in zone 3.

Changes in lung volume

During inspiration, the changes in blood flow that occur within the lungs are complex. They are influenced by the changes in a number of parameters including arterial and venous pressures, cardiac output, the calibre of larger vessels, and alveolar pressures.

It seems likely that the calibre of larger vessels within the lung parenchyma is increased during inspiration as their walls are attached to lung tissue. As this tissue is stretched, so these vessels are pulled open and their resistance decreases. Conversely, the elastic tissue within the walls of these vessels causes their calibre to decrease at lower lung volumes, and it is probable that the flow through them is very small if the lung collapses.

Pulmonary capillaries probably tend to collapse at high lung volumes, as the alveoli become larger and clamp the capillaries running between them. A reduction in systemic arterial pressure therefore occurs. To what extent this happens during tidal respiration is not clear.

Hypoxia

Localized hypoxia has the effect of causing constriction of the pulmonary arterioles in that area of lung. The response seems to be strongest as a consequence of alveolar hypoxia, rather than hypoxaemia. It can be demonstrated experimentally in vessels perfused with oxygenated blood and exposed to low alveolar oxygen concentrations. However, hypoxic vasoconstriction seems to be abolished in isolated pulmonary vessels. This suggests a requirement for a vasoconstrictor substance to be released from the alveoli, although no direct evidence for this exists. The localized hypoxic vasoconstriction appears to be independent of the nervous system. However, a more widespread vasoconstriction in response to global hypoxia does appear to be mediated by the sympathetic system. This may contribute to the pulmonary hypertension of generalized hypoxia in altitude sickness or chronic lung disease.

The vasoconstrictor response is not linearly related to oxygen concentration. Changes in the partial pressure of oxygen to about 12 kPa appear to have little effect on vascular tone, whereas a much more marked effect takes place after changes in the region of 5 kPa. Thus localized hypoxic vasoconstriction is unlikely to play an important role in well ventilated areas of lung, but is an important mechanism for diverting blood away from areas of low oxygen concentration such as collapsed lung segments or during partial obstruction of a part of the bronchial tree. These mechanisms thus enhance the matching of ventilation and perfusion.

Localized and general hypoxic vasoconstrictor responses are important in disease such as chronic obstructive airways disease and sleep apnoea. Long term hypoxia can then lead to pulmonary hypertension. This condition can progress eventually to right heart failure (cor pulmonale).

Pharmacological factors

Pulmonary vasodilatation is brought about by antimuscarinics such as atropine and by β-sympathomimetics such as isoprenaline and dobutamine. Vasoconstriction is brought about by α-sympathomimetics such as adrenaline as well as histamine, 5-hydroxytryptamine (5HT) and some of the prostaglandins.

Measurement of pulmonary blood flow

Fick principle

The Fick principle, when applied to the pulmonary circulation, states that the oxygen consumption per minute equals the oxygen added to the pulmonary circulation per minute. This in turn equals the arterio-venous difference in oxygen content multiplied by the total blood flow.

Thus:

$$\dot{V}_{O_2} = \dot{Q}(Ca_{O_2} - Cv_{O_2}),$$

or

$$\dot{Q} = \frac{\dot{V}_{O_2}}{Ca_{O_2} - Cv_{O_2}};$$

Where: $\dot{Q}$ = total pulmonary blood flow

$\dot{V}_{O_2}$ = oxygen consumption per minute

Ca_{O_2} = arterial content of oxygen

Cv_{O_2} = mixed venous content of oxygen

The value of $\dot{V}_{O_2}$ can be calculated from measurements of the oxygen concentration of the expired gas and the minute ventilation: Ca_{O_2} can be measured by obtaining a sample of arterial blood and Cv_{O_2} can be measured from a sample of blood taken from a catheter in the pulmonary artery or right ventricle.

A similar calculation can be performed using carbon dioxide instead of oxygen. This technique has the advantage that in healthy subjects, the Cv_{CO_2} can be estimated from the end tidal CO_2 concentration ($P_{E_{CO_2}}$) (see also Chapter 21) to obtain an approximate result for $\dot{Q}$.

Measurements of the regional distribution of blood flow in the lungs can be made using radioactive xenon dissolved in saline and injected into a peripheral vein. As the dissolved gas reaches the alveoli, it comes out of solution into the alveolar gas. If the subject holds his breath, the xenon tends to accumulate in the parts of the lungs where it was released, and this quantity at different levels in the chest can be estimated by radioactivity counters.

Matching of ventilation and perfusion

To obtain maximum oxygenation of blood, it is necessary for minute ventilation ($\dot{V}$) and blood flow ($\dot{Q}$) to any given area of lung to be as closely matched as possible. The overall ventilation perfusion ratio is usually about 0.8 for the lungs as a whole. If an area of lung has a relatively low ventilation–perfusion ratio then the blood leaving that area will have an oxygen content which is below normal. It is not possible for blood from other areas of the lung to attain oxygen contents much above normal to compensate for this. The reason for this is that haemoglobin is normally already about 97% saturated with oxygen. The blood is therefore capable of carrying only very little extra oxygen whilst air is being breathed.

For carbon dioxide, the situation is rather different. The normal Pa_{CO_2} lies near the middle of the carbon dioxide dissociation curve. Blood from areas with a high ventilation–perfusion ratio has a relatively low carbon dioxide content, but this is compensated for by blood from areas with a low ventilation–perfusion ratio which has a relatively high carbon dioxide content. By altering the minute ventilation, and therefore the ventilation perfusion ratios of all lung units, the Pa_{CO_2} can be quite closely controlled.

Although the overall ventilation–perfusion ratio is usually close to 0.8 for the lungs as a whole it varies at different levels of the lungs. As we have seen, both blood flow and local alveolar ventilation increase from the top of the lungs to the bottom in an upright subject. However, the differences in blood flow between the apices and bases of the lungs are much greater than the differences in ventilation. Thus, at the apices, ventilation exceeds perfusion and the $\dot{V}/\dot{Q}$ ratio is thought to be about 3. Going down the lung, both ventilation and perfusion increase, but perfusion does so at a much greater rate, so that at the base, perfusion exceeds ventilation and the $\dot{V}/\dot{Q}$ ratio is 0.6. Thus, there are areas in the normal lungs where the ventilation–perfusion ratio is less than ideal. These areas are a source of blood with relatively low oxygen content which then mixes with blood from other areas of the lungs. The effect this has on the total oxygen content of arterial blood is negligible in the normal subject,

but in patients with respiratory disease, the area of lungs with an abnormal ventilation–perfusion ratio is increased. This is the *most important* cause of hypoxaemia in these patients.

Shunt and venous admixture

The oxygen content of arterial blood is always less than that which would be predicted from its dissociation curve and the measured or calculated alveolar concentration of oxygen. Only a very small part of this difference is due to the diffusion gradient between the alveoli and the red cells which must exist in order that gaseous exchange can take place. Most of the difference is due to the admixture of blood with a relatively low oxygen content, some of which results from ventilation–perfusion ratio mismatch and some of which is due to shunting.

The term *anatomical shunt* refers to blood which enters the arterial system without passing through ventilated areas of the lung. The main sources of shunting have been mentioned earlier in this chapter. They are the blood from the bronchial circulation which has an oxygen content little above that of mixed venous blood and the thebesian veins which contribute a small amount of blood with a very low oxygen concentration to the arterial circulation.

Shunting causes the oxygen content of arterial blood to be lowered. Clearly the same effect is brought about by the mismatch of ventilation and perfusion. It is possible to express the magnitude of this reduction in oxygen content by calculating the partial pressure of oxygen in the alveoli using the alveolar gas equation (see Chapter 24) and measuring the arterial partial pressure of oxygen. The difference between these two values is known as the alveolar–arterial oxygen gradient. It provides a measure of the degree of shunting and ventilation-perfusion mismatch that is occurring. The value of the alveolar–arterial oxygen tension is of the order of 0.5–2.5 kPa in normal subjects breathing air.

Venous admixture or physiological shunt, is another concept used to quantify the degree of shunting and ventilation-perfusion mismatch. If there were no shunt and no ventilation-perfusion mismatch, the arterial blood leaving the lungs would have an oxygen content which would be very close to that predicted from its alveolar partial pressure and the oxygen dissociation curve. The venous admixture is that volume of mixed venous blood that would be required to be added to such 'ideal' arterial blood in order to reduce the oxygen content to the level which is actually found in arterial blood samples. If there is little ventilation–perfusion mismatch, then the venous admixture will be similar to the volume of shunted blood. However, these values will not be identical, since the oxygen content of the shunted blood is not the same as that of mixed venous blood. Furthermore, the venous admixture may also include a component due to ventilation-perfusion mismatch should this be significant. The blood draining even underventilated alveoli has an oxygen content which is usually significantly higher than that of mixed venous blood. Hence the volume of the venous admixture due to ventilation-perfusion mismatch will bear little relationship to the true volume of blood actually affected by the mismatch. Nevertheless, a calculation of the venous admixture provides a useful aid to selecting appropriate oxygen therapy and monitoring the progress of treatment.

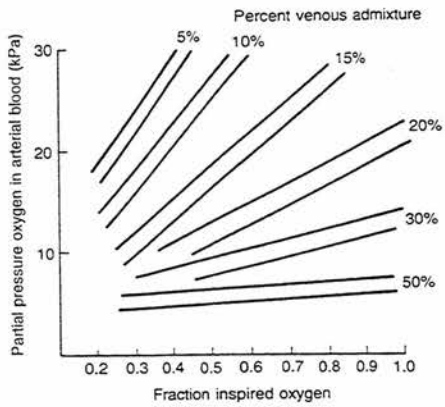

Figure 23.5 Diagram used to estimate venous admixture. By plotting the arterial tension of oxygen for any given inspired fraction of oxygen the percentage of cardiac output that is venous admixture is obtained Adapted from Benatar, Hewlett and Nunn (1973) *British Journal of Anaesthesia*, **45**, 711.

Venous admixture is usually quoted as a percentage of cardiac output. It may be calculated from the following equation:

$$\frac{\dot{Q}_s}{\dot{Q}} = \frac{Cc_{O_2} - Ca_{O_2}}{Cc_{O_2} - Cv_{O_2}}$$

where $\dot{Q}_s$ is the venous admixture, $\dot{Q}$ is the cardiac output, Cv_{O2} is the oxygen content of the venous blood and Cc_{O2} is the oxygen content of capillaries of blood draining alveoli. If the venous admixture is to include a component due to ventilation-perfusion mismatch, then Cc_{O2} is calculated from the alveolar gas equation and the oxygen dissociation curve.

In practice, the venous admixture for a given inspired oxygen concentration and arterial tension is obtained by reference to a graph such as that shown in Fig. 23.5, usually referred to as a 'shunt diagram'. The bands on this graph are sufficiently wide to encompass a range of normal values of carbon dioxide tension and haemoglobin concentrations.

The venous admixture in normal subjects is usually about 5% of cardiac output but rises with age. Higher values usually result from ventilation–perfusion mismatch resulting from lung disease, or occasionally congenital heart disease. By estimating the venous admixture, it is possible quickly to find the optimum inspired oxygen concentration to bring arterial oxygen tension to normal (For large values of venous admixture this is not always possible). In addition, by monitoring changes in the venous admixture, it is possible to follow the progress of treatment.

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(1996)

Chapter 24

Ventilation and the control of breathing

A.S. Davies and C. Moores

The work of breathing

The evolutionary development of lungs as blind-ended internal organs of respiration necessitated the development of a mechanism of tidal ventilation. In man, at rest this ventilation comprises about 12 breaths a minute each of about 0.5 litres, making up a minute ventilation of 6 l min^{-1}. This tidal movement of air can only take place along an unobstructed airway if a difference in pressure exists between its ends (lips, nose and alveoli). The difference in pressure may be provided by the respiratory muscles or, in clinical situations by mechanical means. In quiet breathing, the inspiratory muscles, of which the diaphragm is the most important, but also the external intercostal muscles, provide the energy for inspiration and, by storing energy in the elastic tissue of the lungs, expiration. At minute ventilations in excess of about 40 l min^{-1}, in the presence of expiratory resistance, during phonation and during expulsive efforts, the expiratory muscles are brought into action, first the rectus abdominis, external and internal obliques and transversus abdominis. At even higher minute ventilations the accessory muscles including the scalenes, sternomastoids and extensors of the vertebral column are recruited.

In a resting healthy subject the work of breathing accounts for only about 2% of total metabolism. Part of the energy used to bring about inspiration is 'recovered' as expiration is brought about by elastic recoil. The efficiency of normal breathing is reduced as minute ventilation is increased. It eventually reaches a level where any further increase consumes more oxygen than it provides. This limit is reached much earlier in many forms of respiratory disease where it may limit exercise tolerance. However there is no such law of diminishing returns during artificial ventilation as energy is then provided by an external source. The volume changes that can be produced by the respiratory muscles are shown in Fig. 24.1.

The work of breathing is minimal when inspiration starts from the Functional Residual Capacity (FRC). For any given

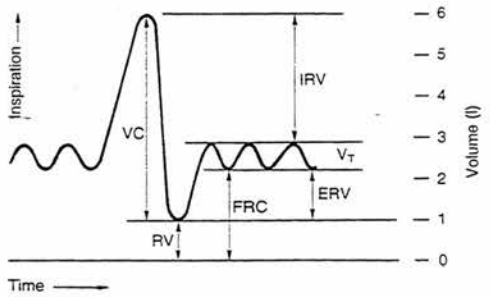

Figure 24.1 Lung volumes that can be measured with a spirometer include: tidal volume (V_T), vital capacity (VC), inspiratory reserve volume (IRV) and expiratory reserve volume (ERV). Because residual volume (RV) cannot be expired, it and functional residual capacity (FRC) cannot be measured by direct spirometry.

minute ventilation it depends on the pattern of breathing and the mechanical properties of the lungs, chest wall and any apparatus through which the subject may be breathing. There are two main sources of impedance to ventilation of the healthy lungs. First, there is the elastic recoil of the lungs and chest wall. Second there is a frictional resistance to gas flow in the airways. Work against elastic resistance is increased when breathing is slow and deep. That against flow resistance is increased when breathing is rapid and shallow. We seem to select a pattern which minimizes respiratory work, probably by monitoring feedback from receptors in the lungs and chest wall.

If total ventilation suddenly changes to a new maintained value the values of the arterial P_{O_2} and P_{CO_2} (Pa_{O_2} and Pa_{CO_2}) change in different and informative ways. Arterial P_{O_2}

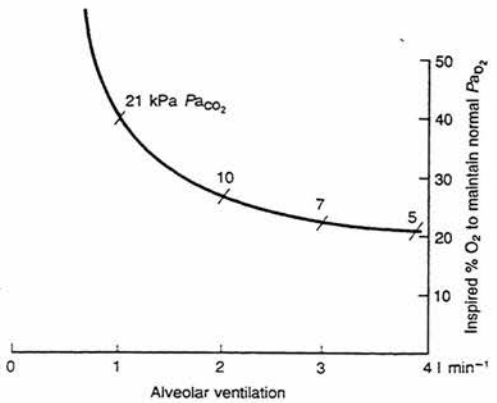

Figure 24.2 Percent inspired O_2 required to maintain normal Pa_{O_2} at different alveolar ventilations. Pa_{CO_2} at each ventilation is shown.

reaches 50% of its new value in less than a minute, P_{CO_2} takes a half-time of 15 minutes to reach its steady state. These differences reflect the different body stores of the two gases. Changes in ventilation have diametrically opposite effects on Pa_{O_2} and Pa_{CO_2} as can be seen in the familiar clinical situation where O_2 is added to the inspired air of a patient with progressively impaired minute ventilation. Despite achieving normal Pa_{O_2} by this method Pa_{CO_2} inexorably rises (Fig. 24.2). These changes are predicted by the alveolar gas equation (see below).

Ventilation can be imposed on a subject's lungs, clinically usually by intermittent positive pressure (IPPV) applied via a cuffed endotracheal tube. The major uses of this treatment are during:

1. Resuscitation and intensive care;
2. Paralysis in disease and anaesthesia;
3. Chronic ventilatory failure.

The characteristics of the imposed ventilating cycle (tidal volume, flow rates, inspiratory/expiratory ratio) can usually be changed by controls on the ventilator to effect optimal ventilation. Anaesthesia normally produces a reduction in functional residual capacity (FRC) and hence lung function (see Chapter 60). To counteract this many ventilators provide positive end-expiratory pressure (PEEP) to maintain the patency of the airways. This is not without its own drawbacks, the most important of which is perhaps the reduction in cardiac output produced by the rise in intrapleural pressure, which obstructs venous filling of the heart.

Dead space

Essentially all gas exchange takes place at the alveolar surface. The airways that connect this surface to the atmosphere make up the *anatomical dead space*. It can be seen from Fig. 24.3 that this space fills with expired air at the

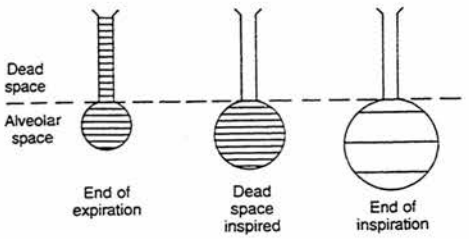

Figure 24.3 The relationship of anatomical dead space to an inspired breath. It can be seen that dead space fills with 'used' alveolar air at the end of expiration and this is first inspired during the next inspiration.

end of each expiration and this is the first gas drawn into the respiratory regions of the lungs. Because the respiratory region is the only part of the lungs that contributes CO_2 to the expired air, the volume of a subject's dead space can be measured as that volume of air expired before CO_2 appears. This volume in millilitres is roughly equal to the subject's weight in pounds. Dead space of any associated measurement apparatus adds to the dead space of the subject. In contrast, endotracheal intubation reduces the functional volume of the upper airways, and thereby bypasses about half of total anatomical dead space. The effect of 'streamlining' of inspired gas to form a 'core' in the centre of the airways penetrating deep into the lungs and the churning action of the heart reduces the measured anatomical dead space during hypoventilation. It is reported that some inspired gas can be found in the alveoli of subjects with tidal volumes as low as 60 ml.

If the volume of that part of the respiratory region of the lung which is ventilated but not perfused is added to the anatomical dead space this makes up the *physiological dead space*, which is the functionally ineffective part of ventilation. This can be defined by the Bohr Mixing equation where

$$\text{Physiological dead space} = \text{Tidal volume} \times \frac{(\text{arterial } P_{CO_2} - \text{mixed expired } P_{CO_2})}{(\text{arterial } P_{CO_2} - \text{inspired } P_{CO_2})}$$

and will have the units of volume.

The functionally effective part of breathing, *alveolar ventilation*; can therefore be described as:

V_A = respiratory frequency × (tidal volume − physiological dead space)

The concentration of a gas in the alveoli is determined by a balance between its rate of uptake (for a gas such as O_2) or production (for CO_2) and alveolar ventilation. The relationship between alveolar ventilation and alveolar gas composition is described by *the universal alveolar gas equation*

$$\text{Alveolar concentration} = \text{Inspired concentration} \pm \frac{\text{Output or uptake of gas}}{\text{Alveolar ventilation}}$$

(On the right-hand side of the equation + is used for gases being evolved, e.g. CO_2 and − is used for gases being absorbed e.g. O_2)

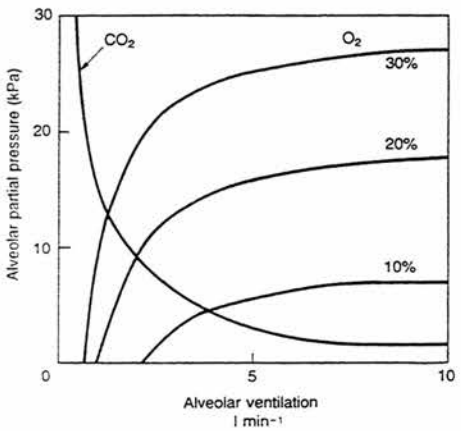

Figure 24.4 The relationship between alveolar partial pressure of a gas and alveolar ventilation as predicted by the alveolar air equation. Three concentrations of inspired O_2 are shown.

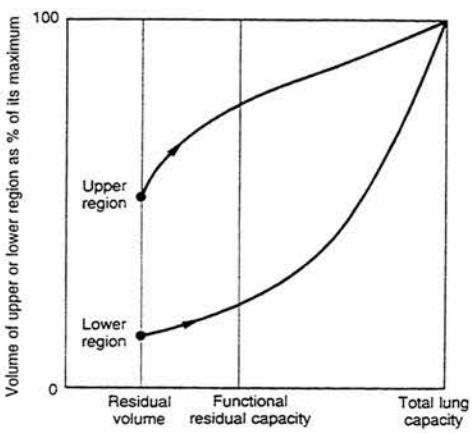

Figure 24.5 Effect of a gradient of pressure due to gravity on the volume of upper and lower regions of the lung at different total lung volumes. The *change* of volume, and therefore ventilation, from functional residual capacity to total lung capacity is greater for the lower region. Adapted from West, J.B. (1985). *Ventilation/blood flow and gas exchange*. 4th edn. Blackwell, Oxford.

The alveolar gas equation takes the form of a rectangular hyperbola and is shown in Fig. 24.4 for O_2 and CO_2. The horizontal asymptote represents the inhaled concentration of the gas and the vertical asymptote represents values obtained with no ventilation.

Before penetrating very far into the lungs an inspired breath is modified by the structure of the bronchial tree. Axial streaming and turbulence both 'smear' the concentration gradient along the airway. This *stratification* of inspired gas is most important in diseases of the lung like emphysema where changes in the geometry of the airways have already made diffusion distances unacceptably long. There are, in addition to stratification, regional differences in alveolar gas composition caused by differences in regional ventilation and differences in blood flow causing different rates of exchange of O_2 and CO_2.

Regional ventilation

The effects of gravity

In the upright human chest, intrapleural pressure increases by about 0.025 kPa (0.25 cmH_2O) for every centimetre moved towards the base of the lungs. This additional pressure on the bases of the lungs causes them to be more effectively 'purged' of residual gas during expiration and in a single breath they can therefore utilize a greater percentage of their potential ventilation. This effect was first investigated by West (1962) using excised lungs surrounded by foam to mimic the gradient of intrapleural pressure. It means that the base of the lungs are about 50% better ventilated than the apex (Fig. 24.5).

Differences in mechanical *time constants* of different parts of the lung also have a profound effect on differences in ventilation. Because its inflation follows an exponential curve, the time constant of the whole or part of a lung can be considered to be the time for inflation to 63% of the final volume attained if inflation is prolonged indefinitely. It is mathematically described as the product of the compliance and airway resistance of the region in question.

The distribution of time constants is narrower in healthy subjects than in patients with lung disease. A narrow distribution of time constants results in a distribution of inspired gas which is independent of breathing frequency and an absence of any internal redistribution of air between different regions of the lung. However, if different parts of the lung have widely different time constants, as happens in some pulmonary diseases the distribution of inspired gas to different lung regions becomes dependent on the pattern of breathing and there will additionally be an internal redistribution of air between different regions of the lung. Individual patients show the same distribution of $\dot{V}$ whether spontaneously breathing or artificially ventilated. This is surprising in view of the difference in movements of the thorax exhibited in the two states.

A uniformity of inspired gas distribution in the lungs implies a uniform gas mixing and an optimal washing in or out of therapeutic substances such as administered oxygen or volatile anaesthetics. Conversely, regions of the lung with short time constants fill and empty rapidly, and contribute their portion of an expired breath before those regions with long time constants. Unacceptably large differences in time constants are frequently a manifestation of lung disease and can be detected by single and multiple breath washout tests.

The single breath washout

In this a single breath of 100% O_2 is taken, diluting the residual N_2 in the subsequent expiration. If plotted against the volume expired N_2 concentration in the expired air through the time course of an even exhalation has the form shown in Fig. 24.6.

In a healthy subject, after clearing the dead space (phase 1), the fraction of N_2 F_{N_2} rises rapidly (phase 2) to an alveolar plateau (phase 3) which has a slight slope. The latter is due in part to O_2 being consumed more rapidly than CO_2 is produced throughout the manoeuvre. There is a slight upturn of the curve (phase 4) as the lung approaches residual volume. This is due to airway closure of the lower regions of the lung, which causes a greater proportion of the expirate to be contributed by the upper regions which have received a higher F_{N_2}. The lung volume at which this starts to happen is the critical closing volume (CCV) which may occur at higher volumes in lung disease. If there is a great disparity in time constants between regions of the lung, sequential emptying takes place with different regions contributing their different compositions of expirate at different times.

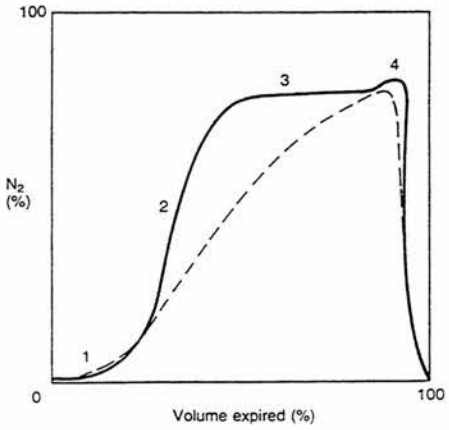

Figure 24.6 Single breath washout curve of nitrogen from a healthy subject and (broken line) a patient with a disparity of time constants produced by lung disease. In that curve the four phases described in the text are no longer clearly distinguishable.

Multiple breath washout

Another way of measuring the uniformity of distribution of inspired gas and gas mixing in the lung is to measure the rate at which a tracer gas is washed out over a number of breaths. In practice, the subject repeatedly inhales pure O_2 and exhales through a N_2 meter which records the concentration of residual N_2 as it is washed out with each expiration. Theoretically the concentration of N_2 should fall in an exponential curve, never quite reaching zero. Thus, if the log of N_2 concentration is plotted against the number of breaths taken for healthy lungs, the graph is almost a straight line, with all the N_2 effectively washed out in 5-7 minutes (Fig. 24.7).

In diseases which cause a non-uniform distribution of ventilation, the graph becomes less steep, more non-linear and the time for any degree of washout is prolonged. Increasing age also contributes to inequalities of gas distribution and mixing, probably by exaggerating differences in mechanical properties due to degenerative changes: so does posture because of the influence of gravity, and airway smooth muscle tone, by changing regional mechanical properties. However, increased tidal volume or the frequency of breathing have little effect because they do not alter the time constants of the regions of the lung. On the other hand, reducing lung volume does affect distribution and mixing if it is sufficient to cause airway closure.

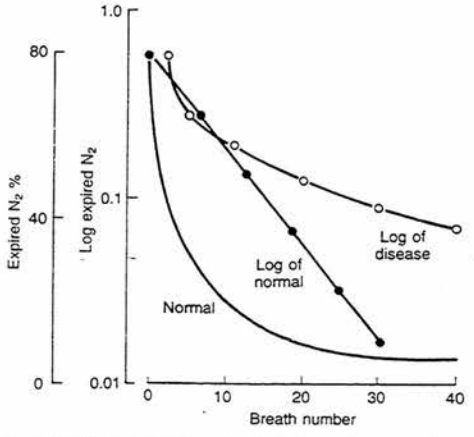

Figure 24.7 Multiple breath washout of nitrogen by oxygen from normal and diseased lungs having less uniformity of gas mixing. Note the non-linear log plot of the slower washout from the diseased lungs.

Pulmonary blood flow

This chapter describes ventilation of the lungs, its distribution and control. However, this ventilation would be futile without appropriate perfusion of the ventilated respiratory surface. Maldistribution of perfusion is the commonest cause of impaired oxygenation of the blood and is usually greater than maldistribution of ventilation. These differences are generally due to the effect of gravity on the more dense blood. Blood flow per unit lung volume therefore, like ventilation, increases from apex to base of the lungs. (See Chapter 23).

Neural control of ventilation

Control of respiratory muscles

The diaphragm possesses few muscle spindles; it is controlled almost entirely by direct α-motoneurones which are unusual

in that they lack Renshaw cells to cut short the after-discharge of activity. The control of the other respiratory muscles, the intercostals and abdominals, more closely resembles that found in most other skeletal muscles and accordingly these have a normal complement of spindles. This may reflect their twofold, respiratory and postural functions. The external intercostals drive inspiration and the internal intercostals cause expiration. Both are active in quiet breathing. The expiratory abdominal muscles only act in forced expiration as occurs in exercise. The respiratory muscles of the thorax and abdomen are also involved in posture and locomotion. It is remarkable how they are under sufficiently fine control to bring about the modulation of speech even under extremes of posture or during locomotion.

Control of breathing

The process of ventilating the lungs, breathing, is unique in that, unlike other automatic systems in the body, it can be used for subsidiary important voluntary actions such as talking, or to assist in tasks such as weight lifting or playing musical instruments. To more easily understand the control of ventilation we can arbitrarily divide it into *chemical control* and *neural control*.

Chemical control ensures homeostasis of O_2, CO_2, and $[H^+]$ by controlling minute ventilation on a relatively long timescale (minute to minute). The minute ventilation ($\dot{V}_E$) is the product of tidal volume (V_T) and frequency of breathing (f):

$$\dot{V}_E = V_T \times f$$

It can be seen that a particular level of ventilation could be made up of an infinite number of combinations of tidal volume and frequency. Individuals 'choose' an optimal pattern of breathing to minimize the amount of work required and the tension in respiratory muscles to produce a particular ventilation. This optimal pattern depends on the ventilation required and the mechanical properties of the lungs. The pattern is determined by a rhythm generator in the brain stem, the output of which is modulated by a number of afferent inputs. By these mechanisms, the motor output in the phrenic and intercostal nerves, which control activity in the major muscles of respiration, the diaphragm and intercostal muscles, can be controlled with a very brief latency (fractions of a second).

The rhythm generator

The pons and medulla of the brain contain the structures that give rise to the basic pattern of breathing. Thus a remarkably normal pattern of breathing persists even if the medulla is isolated from higher centres. Breathing is only abolished by transection between the medulla and spinal cord. (There is some evidence that generators of respiratory rhythm exist in the spinal cord, but to all intents and purposes these can be neglected). The nature of the functional circuitry of neurones in the rhythm generator has yet to be completely agreed. Nevertheless, it is likely that:

1. The activity of neurones bringing about inspiration is synchronized by a linking of excitatory connections. The same is true for neurones bringing about expiration.

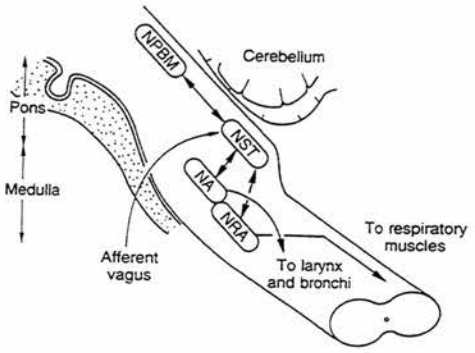

Figure 24.8 The dorsal respiratory group (the nucleus of the tractus solitarius, NST) and the ventral respiratory group (the nucleus ambiguus, NA, and the nucleus retroambigualis, NRA) of the medulla shown together with their connections on one side only.

2. In normal breathing, the duration of activity of inspiratory and expiratory neurones is limited, at least in part, by self-inhibiting activity.
3. The expiratory neurone pool, if active in eupnoea, does not reach a level which activates expiratory muscles.

Medullary respiratory neurones

Patients with damaged brainstems sometimes present with slow deep unmodulated patterns of breathing or even apnoea. From observations in these patients and recording in experimental animals two functionally distinct groups of neurones have been found in the medulla (Fig. 24.8).

The dorsal respiratory group (DRG)

This is found in the region of the tractus solitarius. The group of cells is largely made up of inspiratory neurones. It may be the pacemaker from which the basic rhythm of breathing originates. Activity from mechanoreceptors and chemoreceptors affect this region to modulate breathing.

The ventral respiratory group

This is found rostrally in the nucleus ambiguus and caudally in the nucleus retroambigualis. This functional group of cells contains both inspiratory and expiratory neurones. The rostral part innervates ipsilateral accessory muscles of respiration. The caudal part innervates the contralateral diaphragm, the contralateral expiratory intercostal and abdominal muscles and the ipsilateral and contralateral inspiratory intercostal muscles. It must be stressed that these 'groups' constitute functional categories of cells which are found in higher concentrations in the regions indicated rather than discrete anatomical entities. Transection experiments which led to the understanding of the complementary action of the upper pons and the vagi have also given rise to the erroneous idea of discrete anatomical centres rather than functional groups.

Voluntary and automatic breathing

In man, there is a clear separation of the voluntary and automatic neural pathways that control breathing. Voluntary pathways from higher centres bypass the respiratory groups of neurones in the brainstem and descend in the pyramidal tracts. Control by way of these pathways is always bilateral; for example, we cannot contract half our diaphragm. These nerve fibres may originate in the motor cortex. If these pathways are destroyed, for example by a stroke, automatic breathing in response to homeostatic demands may be normal but breathing cannot be voluntarily controlled. For example the patient will cough reflexly on laryngeal stimulation but not on command. Conversely, severe brain stem damage which spares the pyramidal tracts can result in the opposite functional dissociation known as Ondine's curse. In such a situation, the patient can breathe deliberately but loses the capacity for automatic breathing and requires artificial ventilation during sleep. In the spinal cord, the voluntary upper motor neurone pathways from the cortex are found in the lateral areas and the automatic pathways are more anterior near the origins of the ventral roots.

Chemical control of ventilation

It is quite artificial to separate control of ventilation into neural and chemical control, if only because the elements involved in chemical control are anatomically neural tissue. However, such a separation is traditional because it is convenient for ease of description.

The sensors involved in chemical control of breathing are called chemoreceptors, and in addition to their categorization by location into central and peripheral receptors it is possible to categorize them into those that are sensitive to oxygen lack (hypoxia) and those sensitive to carbon dioxide excess (hypercapnia).

The role of hypoxia

The *peripheral chemoreceptors* sense hypoxia, or more precisely hypoxaemia, and hypercapnia. They are unusual in being stimulated by hypoxia which depresses the activity of most (especially excitable) cells. The major peripheral chemoreceptors in man are the carotid bodies and aortic bodies. these are small (5 mm diameter) nodes of glomus tissue. There are two carotid bodies; these are situated close to the bifurcation of the common carotid arteries into the internal and external branches, from which they receive their blood supply. They send their information to the brain in the sinus branches of the glossopharyngeal nerves. The aortic bodies, less important in man, are variable in number, and are scattered around the aortic arch; they send their information in the vagus nerve.

The peripheral chemoreceptors are made up of type I and type II cells and afferent and efferent nerve fibres. The type I cells appear to be the site of stimulation by hypoxia and release a transmitter which stimulates afferent nerve endings. The type II cells resemble supporting or glial cells. The efferent nerves to the peripheral chemoreceptors modify their sensitivity to hypoxia, in part by modifying the blood flow through them.

The carotid bodies have an enormous blood flow through them. Weight for weight this is 40 times that of the brain!

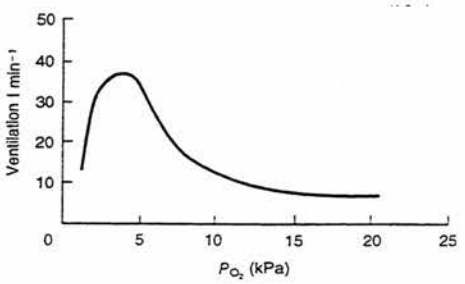

Figure 24.9 Change in ventilation with change in arterial Po_2 at normal (5.2 kPa) Pa_{CO2}.

Their metabolic rate is also very high. As the glomus cells are not neurons themselves the question has arisen as to how they stimulate the afferent nerve fibres in the sinus nerve as a consequence of hypoxia. Their blood flow and metabolic characteristics have led to the suggestion that their specific response to hypoxia is a depression of metabolism with an accumulation of metabolites which reach a local concentration capable of chemically exciting the afferent nerve endings. In anaemic hypoxia there is no chemoreceptor mediated stimulation of breathing and this may be explained by the fact that the maintained high blood flow washes the metabolites away. In hypotension, or circulatory depression causing stagnant hypoxia metabolite build-up occurs and acts as a potent stimulus to breathing.

Breathing is remarkably insensitive to hypoxia until the Pa_{O2} is reduced to about half its normal value (Fig 24.9). As well as stimulating breathing, peripheral chemoreceptor activity increases heart rate, constricts peripheral blood vessels (except in the skin) and increases activity in the adrenal medulla. There is also an elevation in blood pressure. Peripheral chemoreceptors respond to hypercapnia more rapidly than their central counterparts and can maintain their response even when the central chemoreceptors are down regulated.

The peripheral chemoreceptors are also stimulated by an increased arterial hydrogen ion concentration (reduced pH) of respiratory or metabolic origin and also directly by an increase in Pa_{CO2}. Their sensitivity to the latter is, however, only about one tenth of that of the central chemoreceptors.

Although important as the only sites in the body where hypoxia can stimulate breathing the peripheral chemoreceptors' relative insensitivity is made worse by a tendency for hypoxic driven increases in ventilation to wash out carbon dioxide and so reduce its stimulating effect on breathing. If this 'hypocapnic brake' were not present the effects of hypoxia on ventilation would be up to 10 times greater.

Clinical correlates

Human natives of high altitude have a blunted response to hypoxia, which is not reversed even after several years residence at sea-level. Other species which live at high altitude maintain a normal hypoxic response and this species difference may reflect the difference in time, in evolutionary terms, the species have spent at altitude. An interesting, and

unexplained, aspect of this blunting is its alleviation in pregnant high altitude women.

Bronchitic patients with serious respiratory failure show a similar lack of response to their indigenous hypoxaemia and any further imposed hypoxia. It is highly probable that this unfortunate situation is the consequence of changes of sensitivity of their carotid bodies. A method of returning their sensitivity to normal, and so promoting an appropriate level of ventilation would be of clinical value.

The unpleasant situation of dyspnoea, perhaps the most common symptom in chest medicine, has been associated with the carotid body. Surgical excision of one or both carotid bodies has been carried out in a number of chronic obstructive airways disease and asthmatic patients and in many of the cases existing hyperventilation was reduced.

The role of hypercapnia

Carbon dioxide accounts for the greater part of the chemical drive to breathe under resting conditions, and the *central chemoreceptors* located in the medulla of the brain account for 75% of that drive. Unlike the response to hypoxia any increase in the level of inhaled CO_2 (normally 0.03% in fresh air) causes a near linear increase in ventilation (Fig 24.10).

The response to a stepwise increase in inhaled CO_2 begins within 15 s but takes about 5 minutes to reach equilibrium. This time course is the result of CO_2 stimulating at two sites. The peripheral chemoreceptors are reached very rapidly. There are also the central chemoreceptors situated just (500 μm) below the surface of the ventrolateral medulla of the brain. These central receptors contribute at least 75% of the total response, but to reach them, CO_2 has to cross the blood–brain barrier. It is assumed that the neural elements that make up the central chemoreceptors are influenced by changes in composition of the interstitial fluid that surrounds them. The composition of this fluid and that of the cerebrospinal fluid, which also influences it, is governed by the activity of the blood–brain barrier. This controls the movement of ions but not of O_2 and CO_2 which readily pass between plasma and cerebrospinal fluid. Thus CO_2 rapidly diffuses from arterial blood across the blood–brain barrier into the interstitial and cerebrospinal fluids. It then hydrates to form H^+ which is probably the specific stimulus to the central chemoreceptors.

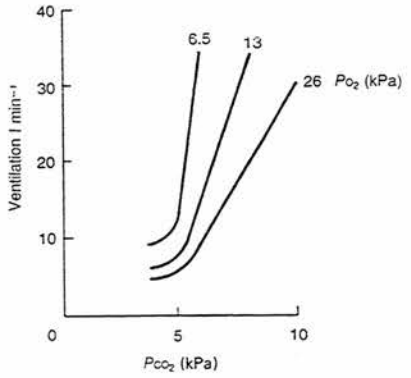

Figure 24.10 Change in ventilation with change in arterial P_{CO_2} at three levels of $P_{a_{O_2}}$.

The movements of ions, particularly HCO_3^- across the blood–brain barrier, are homeostatic. They tend to return cerebrospinal fluid pH to normal in a day or two. In chronic lung disease, with increased arterial P_{CO_2}, this compensatory response is inappropriate, as it blunts the required increase in ventilation which offsets impaired lung function. The influence of the blood–brain barrier on HCO_3^- ions was once thought to be an active process. In fact, it may be due to passive distribution of ions. H^+ does not readily pass the blood–brain barrier and so changes in blood $[H^+]$ do not affect central chemoreceptors if arterial P_{CO_2} is constant.

Other afferent inputs can maintain breathing in the absence of stimulation of the central chemoreceptors by CO_2. Thus Fig. 24.10 shows that reductions in arterial P_{CO_2} by hyperventilation, for example, do not reduce the subject's spontaneous minute ventilation below a certain basic level.

Asphyxia

The ventilation resulting from asphyxia (hypoxia + hypercapnia) is not the simple sum of the changes in ventilation brought about by the changes in P_{CO_2}, P_{O_2} and pH brought about by each of the components. Changes in exercise level complicate the matter further and independently.

The need to describe '*gestalt*' control of minute ventilation was perhaps first expressed by Gray in 1945 in the formula

$$VR = 0.22\ [H^+] + 0.262\ P_{CO_2} - 18 + \frac{105}{(10^{0.038}\ P_{O_2})}$$

Where VR is the ratio of the stimulated alveolar ventilation to its rest value. The values and relationships in Gray's formula have been modified and challenged by more recent research, but it still emphasizes the complex interaction of the factors which determine minute ventilation such as the 'hypocapnic brake' which has already been mentioned and the synergistic interaction of P_{O_2} and P_{CO_2} in asphyxia illustrated in Fig. 24.10.

Reflex control of breathing

The basic pattern of breathing described above is modified, mainly by activity of receptors in the lungs, to produce a pattern which is energy-efficient and which reflects the mechanical properties of the lungs. Many types of receptor have been postulated to exist in the lungs. However, it seems now that most reflex control of breathing can be explained in terms of pulmonary stretch receptors, rapidly adapting receptors and C-fibre receptors.

Pulmonary stretch receptors

Slowly adapting receptors sensitive to stretch are found in the smooth muscle of the trachea and main bronchi and so are affected by bronchial tone. They respond to stretch of the airways and so increase their frequency of discharge during inspiration. Many are tonically active during the

expiratory pause of normal breathing. It has been postulated that, at least in animals, the increasing activity of these receptors during inspiration reaches a level which operates an 'off switch' in the respiratory complex of the brain that terminates inspiration. The action of these receptors is most clearly seen in the Hering–Breuer inflation reflex in which respiratory effort is inhibited, in anaesthetized animals, by inflation of their lungs. Cutting the vagi abolishes this reflex, identifying its origin. However, such a vagotomized animal continues to breathe without much change in its minute volume at rest. Since stretch receptors are affected by bronchial tone they are affected by changes in lung compliance and airway resistance. In anaesthetized animals, they can be seen to modify the pattern of breathing to maintain the efficiency of this activity when compliance or resistance changes.

The role of stretch receptors in unanaesthetized man is probably different from that in anaesthetized animals. Large inflations (over 1 litre) are required to evoke the Hering–Breuer reflex. The response of anaesthetized subjects to an imposed expiratory resistance is quite different from that of animals and can be demonstrated quite easily by any anaesthetist. Anaesthetized patients respond to increased expiratory resistance, which maintains lung inflation, not with an absence of inspiratory effort but with increased inspiratory force. The latter persists until lung volume reaches a point where the increased elastic recoil is sufficient to overcome the expiratory resistance and ventilation is thus maintained. Nevertheless stretch receptors are present in human lungs, their activity has been recorded in the human vagus nerve and the Hering–Breuer reflex is present in man during sleep. Increased pulmonary venous pressure increases stretch receptor activity and CO_2 inhibits their activity.

Rapidly adapting pulmonary receptors

These receptors consist of free nerve endings close to the surface of the airway epithelium. They are concentrated at the divisions of the airways. These receptors have also been termed *irritant receptors* as they can be stimulated by irritants such as cigarette smoke or ammonia gas, or *deflation receptors* as pneumothorax strongly stimulates them to contribute a major part of the tachypnoea which makes up the deflation reflex. Neither irritants of the type mentioned nor pneumothorax constitute normal, physiological, stimuli and so the term rapidly adapting receptors is the best description of this type of receptor. Thus, the usual physiological stimuli of these receptors are the rate of change of volume of the lungs, which is related to air-flow, and collapse of the lung. Rapidly adapting pulmonary receptors produce two apparently incompatible reflex effects. Their activity produces both rapid shallow breathing, mainly by a reduction in the expiratory duration, and occasional deep sighs or augmented breaths which occur every 5–15 minutes in man and which serve to reverse the slow collapse which develops during quiet breathing. These two very different patterns of breathing can co-exist because each augmented breath seems to be followed by a refractory period of several minutes during which time another cannot be elicited.

Head's paradoxical reflex occurs when the lungs of an experimental animal, in which neural transmission in the vagi is partly blocked, are inflated. Rather than breathing ceasing (the usual Hering–Breuer inflation reflex) the animal now takes a deep breath. This, and the 'gasp reflex' that can be elicited by inflating the lungs of newborn infants and in patients who have received sufficient opiates to reduce respiratory frequency significantly, are probably due to rapidly adapting receptor activity.

It has recently been shown that rapidly adapting receptor activity is increased in a number of animal models of human lung disease. It may be that they are in part responsible for the changed patterns of breathing and the sensation of dyspnoea seen in many lung diseases.

As well as changing the pattern of breathing, pulmonary rapidly adapting receptor activity caused bronchial and laryngeal constriction and airway mucus secretion.

C-fibre receptors

Certain groups of nonmyelinated C-fibres in the vagi are presumed to have endings in the bronchial walls and close to the pulmonary capillaries although neither type of ending has yet been identified histologically. The endings near capillaries are alternatively referred to as J- (juxtapulmonary capillary) receptors. They are stimulated by oedema and substances released during lung damage such as bradykinin, histamine and prostaglandins. They elicit the so-called pulmonary chemoreflex of bradycardia, hypotension, rapid shallow breathing or apnoea, bronchoconstriction, airway mucus secretion and inhibition of spinal motoneurones. This barrage of reflex activity has been suggested as a method of restricting exercise but a role for C-fibre receptors in normal quiet breathing has not yet been demonstrated.

Other reflexes

The cough reflex

Mechanical and chemical stimulation of superficial rapidly adapting receptors of the upper airways, particularly the larynx, can produce a cough. This consists of inspiration of an appropriately large volume of air which is compressed by expiratory effort against a closed glottis which is then rapidly opened to allow a high velocity stream of air to remove the irritating material. Cough is an interesting demonstration of overriding mechanisms in the control of breathing. Thus stimulation of a single cough receptor can overpower the activity of all other afferent systems and provoke a cough. It is also interesting that, at least in animals, pulmonary stretch receptors, which do not have any obvious role in the reflex, must be active for a cough to take place.

Swallowing and sniffing

Swallowing immediately inhibits breathing. This is obviously useful in preventing aspiration of food. Stimulation of the nasopharynx can produce a sniff or aspiration reflex, particularly in infants. This enhanced sensitivity is probably related to the infant's need to maintain a nasopharynx clear of milk while suckling. It is also the rationale for obstetricians clearing the mouths of newborn babies before clearing the nose.

The respiratory muscles

Unlike the diaphragm, which has few muscle spindles, the thoracic respiratory muscles have a normal complement of such receptors. These provide a load detecting mechanism operating at a spinal level. This enables changes in compliance or resistance, intrinsic or applied, to be rapidly compensated for by changes in drive to the respiratory muscles.

Pain

It is generally experienced that sharp somatic pain can cause gasping breathing. This differs from visceral pain. It is reported that distension of the intestine, gallbladder or bile duct inhibits breathing. Traction on other viscera can cause apnoea.

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Chapter 25

The assessment of respiratory function

A.S. Davies and C. Moores

Why assess respiratory function?

The clinician is able to call upon a number of physiological tests of respiratory function in order to help assess the patient with a respiratory problem. These tests can complement, but not replace, a carefully taken history and an appropriate physical examination. They nevertheless have an important and useful role to play.

Before an operation, assessment of respiratory function allows existing therapy to be optimized, and a 'baseline' established to which later measurements of function can be compared. The results of this assessment may influence the anaesthetic and surgical management of the patient and may dictate the type of after-care which the patient may receive. For example, decisions concerning postoperative oxygen therapy, physiotherapy and even elective ventilation are often made before surgery on the basis of preoperative assessments.

In critical care, tests of respiratory function play a vital role in monitoring treatments such as artificial ventilation as well as in charting the clinical progress of the patient.

In this chapter, simple tests of lung function which can be routinely carried out are described first, followed by some less commonly encountered procedures such as might be found in well-equipped respiratory laboratories. Finally, tests of respiratory function more usually confined to the practice of anaesthetics and to critical care medicine are covered. Changes in respiratory measurements, seen in common clinical conditions, are summarized in Table 25.1.

Simple tests of respiratory function

Spirometry

Tidal volume, inspiratory reserve volume, expiratory reserve volume and vital capacity are all lung volumes which can be measured directly using a simple spirometer. In clinical practice, it is wise also to have information about dynamic lung function, in particular the resistance of the airways to gas flow.

For this reason it is customary to measure a patient's *forced expiratory volume (FEV)* and *forced expiratory volume in one second (FEV_1)*. This is achieved by using a spirometer able to produce a graph of the volume of expired air against time. The patient is asked to breathe in to total lung capacity and then to expire *as hard and as fast as possible* into the spirometer, until he can force no more air out of his lungs. A graph of the results of such a manoeuvre is shown in Fig. 25.1.

Table 25.1 Changes in respiratory variables in some common clinical conditions

	FVC	*FEV_1*	*FEV_1/FVC*	*TLC*	*Diffusing capacity*	*Pa_{O_2}*	*Pa_{CO_2}*
Chronic bronchitis and emphysema	↓	↓	Normal or ↑	↑	↓	Normal or ↓	Normal or ↑
Pulmonary fibrosis	↓	↓	Normal	↓	↓	Normal or ↓	Normal
Acute asthma	Normal	↓	↓	↑	↓	Normal or ↓	↓ ↑ if severe
Acute pneumonia	Variable	Variable	Variable	↓	↓	↓	↑ if severe

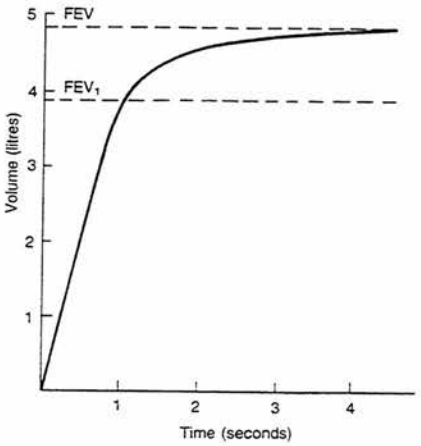

Figure 25.1 Normal spirogram obtained during a forced expiratory manoeuvre. FEV and FEV_1 are marked.

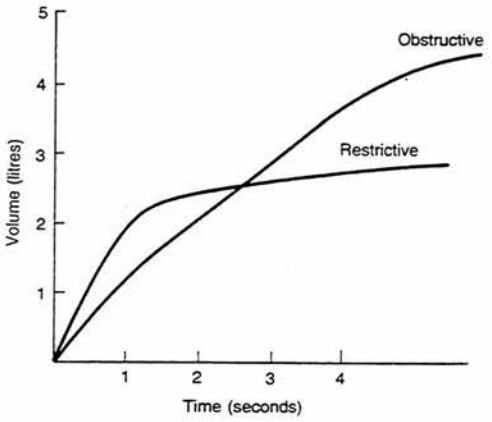

Figure 25.2 Spirogram demonstrating obstructive and restrictive patterns.

The FEV is the total volume of gas that can be expired during a forced expiration. It is numerically very similar to (although in fact slightly less than) the vital capacity and for this reason is sometimes called the *forced vital capacity (FVC)*.

The value of FEV for a given subject is dependent upon such factors as the subject's age, sex and build, as well as lung pathology. When assessing a patient's FEV, it is therefore necessary to compare it with tabulated values obtained from normal subjects of the patient's age and sex.

The FEV_1 is the volume of gas that is expired during the first second of a forced expiration. It is measured directly from the spirometer trace as shown in Fig. 25.1. Its value depends on the value of the FEV for that patient and because of this, it is necessary to calculate the ratio FEV_1/FEV. In normal individuals, this ratio is usually in the range 0.7–0.8.

It is customary to divide respiratory patients into two groups on the basis of their measured FEV and FEV_1/FEV (Fig. 25.2). In patients with a *restrictive pattern*, both the FEV and FEV_1 are reduced by the same proportion, and the FEV_1/FEV is therefore little altered from normal. This type of pattern is seen in diseases which diffusely affect the lung parenchyma causing fibrosis, such as fibrosing alveolitis and sarcoidosis. The fibrous tissue which is deposited in the lungs in these conditions is inelastic and limits the compliance of the lungs, thus reducing both the FEV and FEV_1 proportionately.

On the other hand, patients who have diseases of the airways, such as asthma or chronic obstructive airways disease, frequently present with an *obstructive pattern*. In such patients, the FEV is often normal or modestly reduced, but the FEV_1/FEV is significantly less than normal. This is because airflow is limited by the narrowed airways. It therefore takes longer to exhale a given volume of air than normal. Put another way: during a given time (1 second), a smaller volume of gas can be exhaled. The small reduction in FEV seen in some airways diseases, particularly in chronic obstructive airways disease, belies the fact that total lung capacity is frequently increased in these patients because of destruction of the lung parenchyma. The reason for this increase in capacity is that some of the smaller airways collapse during a forced expiratory manoeuvre as the intrathoracic pressure surrounding them becomes less negative, or even positive, and they have little support from the adjacent, diseased parenchyma. Air is trapped in the part of the respiratory tract distal to such a collapse, and clearly cannot contribute to the FEV. This mechanism of airway collapse on testing is identical to that suffered by such patients when they cough.

Measurements of the FEV and FEV_1/FEV ratio are useful in assessment of the severity and in following the course of respiratory disease. Such measurements also enable treatment to be evaluated. For example, in a patient with airways obstruction, by assessing respiratory function before and 10–15 minutes after the administration of an aerosol of a bronchodilator drug, it is possible to evaluate the reversibility of the airway obstruction.

Expiratory flow

In assessment of respiratory disease, *peak expiratory flow rate (PEFR)* is frequently used as an indicator of respiratory function. The PEFR, as its name suggests, is the maximum rate of air flow that a patient can produce during a forced expiratory manoeuvre. It can be calculated from an FEV curve obtained in the clinic. Its greatest value lies in the fact that it can be measured quickly and cheaply by the patient himself at home. Simple apparatus such as the Wright's Peak Flow Meter is cheap and light, and usually depends on the deflection of a vane by the patient's expired breath to

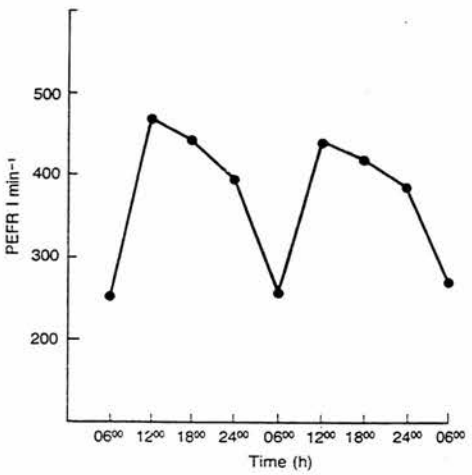

Figure 25.3 Example of diurnal variation of PEFR in an asthmatic patient.

measure flow. Once the patient has been taught to use this apparatus correctly, the results are remarkably reproducible.

The PEFR is a relatively non-specific indicator of respiratory disease and depends, among other things, on the patient's vital capacity, airways obstruction, chest wall compliance and effort. It cannot assist differential diagnosis to the degree that spirometry can, but once a diagnosis is made by other means, it is a useful method of assessing changes in the disease or of monitoring treatment.

Measurement of the PEFR has been particularly valuable in the treatment of bronchial asthma. The severity of this disease frequently undergoes a marked diurnal variation (Fig. 25.3). A patient's condition may appear well controlled during the day at clinic appointments but he may suffer severe attacks of bronchoconstriction at night or in the early morning. The patient who is supplied with a peak flow meter is able to measure his PEFR at home, at different times in the day. This would give a full assessment of the efficacy of treatment during the whole of the 24-hour period. Acute attacks that occur during the night, for example, can then be covered by altering the timing of treatment, or by changing to drugs with a longer duration of action. Patients can also be taught to assess the severity of an individual asthma attack by using a peak flow meter. They can then objectively monitor the efficacy of their treatment and decide when to seek further medical attention.

Diffusing capacity (transfer factor)

The diffusing capacity, or transfer factor of a gas being taken up by a lung is given by the equation

$$D = \frac{\text{rate of uptake of gas}}{\text{alveolar } P_{A_{gas}} - \text{mean pulmonary capillary } Pc_{gas}}$$

Where D is the diffusing capacity, $P_{A_{gas}}$ is the partial pressure of the gas in the alveoli and Pc_{gas} the partial pressure in pulmonary capillary blood.

The diffusing capacity is therefore the rate at which gas is transferred across the alveolar/capillary membrane for a unit difference in partial pressure across that membrane. It is a very sensitive, although non-specific, test of pulmonary function.

A major problem in assessing diffusing capacity is that of measuring the driving partial pressure of the gas from alveoli to blood. Carbon monoxide is used because it has such a high affinity for haemoglobin (about 250 times that of oxygen) that its partial pressure in pulmonary capillary blood can be assumed to be zero if its alveolar concentration is not too high. The diffusing capacity for carbon monoxide thus simplifies to:

$$D_{(CO)} = \frac{\text{rate of uptake of carbon monoxide}}{\text{alveolar } P_{CO}}$$

The diffusing capacity for carbon monoxide can be measured clinically in a number of ways. In the single breath method, a breath containing a small concentration of carbon monoxide is taken, held for 10 seconds and then exhaled. The concentration of carbon monoxide in the inspired and expired gas is measured using infrared spectrometry to estimate values for both the alveolar concentration of carbon monoxide and its rate of uptake from the lungs. In an alternative method, the subject breathes a gas containing a constant small concentration of carbon monoxide over 30 seconds or so until a steady rate of uptake of carbon monoxide is reached, again measured using infrared spectrometry.

As its name suggests, the major factor determining the diffusing capacity is the rate of diffusion of gas across the alveolar/capillary membrane. This will be influenced by other factors including the area of the membrane across which diffusion occurs, the volume of capillary blood, and the time which capillary blood spends in contact with the membrane. For this reason, the more general term *transfer factor* is often used rather than diffusing capacity.

Disease processes can alter the diffusing capacity, or transfer factor, in a number of ways. The alveolar/capillary membrane may become thickened (because of e.g. oedema or fibrosis) directly limiting diffusion. Destruction of lung tissue or ventilation/perfusion abnormalities can lead to a reduced area of membrane over which diffusion can occur and this again can affect the diffusing capacity. As a test of lung function it is therefore relatively non-specific, but its sensitivity is such that it may disclose abnormalities of lung function before abnormalities in spirometry are apparent.

Estimation of functional residual capacity and residual volume

Functional residual capacity and residual volume are two values which are frequently reduced in diseases of the lung parenchyma. They cannot be measured directly by conventional spirometry and so techniques such as helium dilution and total body plethysmography have to be used.

Helium is virtually insoluble in blood. If a subject breathes into a closed spirometer containing a gas mixture including helium, no helium will be lost from the system into the subject's blood. Initially, before the subject starts to breathe

into the spirometer, all the helium will be contained within the spirometer bell. After a period of breathing, the helium will be distributed evenly between the spirometer bell and the subject's lungs and clearly its concentration will be reduced. Since mass = concentration × volume and the total mass of helium in the system remains unchanged it can be seen that

$$C_1 \times V_s = C_2 \times (V_s + \text{FRC})$$

where C_1 and C_2 are the concentrations of helium in the spirometer gas before and after equilibration with gas in the lungs, V_s is the volume of the spirometer bell and FRC is the volume of the lungs at the end of tidal respiration at which point the subject is connected to the spirometer.

It is thus possible to calculate a value for the FRC and by subtracting the expiratory reserve volume from that obtain a value for the RV. In practical terms, when performing the measurement, it is necessary to remove carbon dioxide from the system as the subject breathes, and the volume of oxygen which the subject takes up needs to be either replaced, or accounted for in the calculation of FRC.

In an adaptation of this technique, it is possible to measure FRC using a single breath held for 10 seconds and containing a known concentration of helium. The volume of the breath and the expiratory concentration of helium are measured. This technique has the advantage that it can be combined with a single breath measurement of diffusing capacity for carbon monoxide.

Total body plethysmography, in the form of the 'interrupter method', is another technique which may be used to estimate FRC. The subject sits in a plethysmograph, which is an airtight box, of similar dimensions to a telephone kiosk, and breathes through a mouthpiece (Fig. 25.4). At the end of a tidal breath, a shutter in the subject's mouthpiece closes for a fraction of a second and inspiration is interrupted. As he attempts to inhale against the closed shutter, the volume of gas in his thorax expands, and so its pressure drops. His chest expands and compresses the gas in the plethysmograph around his body so that this volume of gas decreases and its pressure therefore increases. The pressures at the mouthpiece and within the plethysmograph are both measured. Interruption of inspiration is so brief that the subject has virtually no sensation of its taking place.

Initially, the change in volume of the thorax that takes place during the interrupted inspiration is estimated from the change in pressure that occurs within the plethysmograph. The relationship between volume and pressure in the plethysmograph containing the subject is first established by pumping in known volumes of gas and measuring the pressure changes that take place. By applying Boyle's law it is then possible to calculate the change in volume at constant temperature that took place during the attempted inspiration. Boyle's law is then applied to the thorax itself, as follows:

$$P_1\text{FRC} = P_2(\text{FRC} + \Delta V)$$

where P_1 and P_2 are the pressures at the mouthpiece before and after the inspiratory effort has been made and ΔV is the change in volume of the thorax that has already been estimated.

When comparing the values of FRC obtained using the techniques of helium dilution and plethysmography, it is important to consider exactly what each reflects. In the case of helium dilution, it is the volume of the thorax communicating with the mouth that is estimated, whereas in the case of the plethysmograph, it is the total volume of gas within the thorax. In the healthy subject, these two values are very similar, but in the patient with respiratory disease, they may be significantly different. In such patients, collapsed airways may cause pockets of air to be trapped within the lungs. This trapped air is not useful in respiration, since it is not in direct communication with the mouth. It nevertheless undergoes a change in volume in response to changes in the intrathoracic pressure. It is therefore included in the estimate made with the plethysmograph, but not in that made with the helium dilution method.

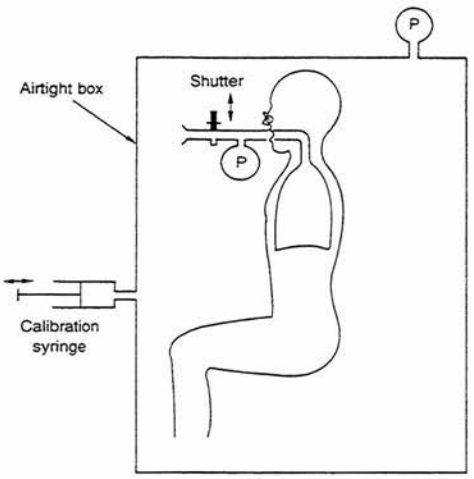

Figure 25.4 The whole-body plethysmograph.

Gas and acid–base determinations

Estimates of arterial blood gas tensions

In the past decade or so, it has become relatively straightforward to estimate the pH and tensions of oxygen and carbon dioxide in blood samples. Typically, a single machine is able to estimate each of the above values from a sample of less than 1 ml of arterial blood. Estimates of the actual and standard bicarbonate and base excess can be calculated from these values.

The system of measuring pH consists of two electrodes, a silver electrode and a HgCl electrode. The HgCl electrode is in contact with the blood sample via a salt bridge, but the silver electrode is separated from the sample by a glass membrane which is selectively permeable to hydrogen ions. A potential difference is set up between the two electrodes and depends upon the concentration of hydrogen ions in the sample able to cross the ion-selective membrane and complete the circuit. A similar arrangement can be used to measure the concentration of CO_2; in this case the H^+-selec-

tive membrane is covered with a bicarbonate buffer, the pH of which depends on the concentration of CO_2 in the sample.

Oxygen tensions are measured using an oxygen electrode consisting of a platinum cathode and a silver anode across which a small voltage is applied. Oxygen enters the electrode chamber through an oxygen permeable membrane and is reduced to hydroxide ions and water at the cathode. At the same time, the silver anode is oxidized to Ag^+ ions. The current that flows is clearly dependent on the concentration of oxygen presenting for reduction at the cathode, and from this current a value for oxygen tension is derived.

The *actual bicarbonate* is the concentration of bicarbonate present in the plasma of a sample. It is not measured directly, but is calculated from the measured values of pH and P_{CO_2} using the Handerson–Hasselbalch equation. The *standard bicarbonate* is the bicarbonate concentration in the plasma of fully oxygenated blood which has been equilibrated with gas having a P_{CO_2} of 5.3 kPa at 37°C. The usefulness of this value is that it estimates what the bicarbonate would be if there were *no respiratory component* to the acidosis/alkalosis. In cases where a respiratory acidosis or alkalosis is suspected from the pH and P_{CO_2} calculation of the standard bicarbonate may reveal a concomitant metabolic upset, or may indicate the degree to which a metabolic compensation has taken place. The *base excess* is the positive or negative amount of strong acid required to titrate 1 litre of blood back to a pH of 7.40 at a P_{CO_2} of 5.3 kPa at 37°C. In practice, it is numerically very close to the difference between the actual and standard bicarbonate.

Measurement of oxygen saturation

The oxygen saturation of arterial blood (Sa_{O2}) is defined by the equation:

$$Sa_{O2} = \frac{\text{oxygen content}}{\text{oxygen capacity}} \times 100$$

where:

oxygen content = the actual amount of O_2 combined with haemoglobin (e.g. ml dl^{-1})

oxygen capacity = the maximum amount of O_2 which can combine with the same amount of haemoglobin (e.g. ml dl^{-1})

Since the units of both are the same the saturation will be ratio with no units. For convenience we multiply the fractional ratio by 100 to give a *percentage*. (N.B. that a percentage % is also a ratio and thus has no units – the % sign is simply a rotated division ÷ sign).

A reliable, non-invasive estimate of Sa_{O2} can now be obtained using spectrometry. This technique is based on the fact that oxyhaemoglobin and deoxyhaemoglobin have differing optical absorption spectra. From a *pulse oximeter*, light of up to four different wavelengths is shone through a piece of tissue, usually the lobe of the ear, and detected by photoelectric cells to give an absorption signal.

To estimate a value of Sa_{O2}, the pulse oximeter electronically separates out the pulsatile part of the absorption signal. The pulsatile part is derived from blood in the arterial side of the vascular tree, before much gaseous exchange with tissues has occurred. By ignoring the rest of the signal, the oximeter is disregarding the absorption that takes place in venous blood and in extravascular tissue.

The mean absorption of the pulsatile part of the signal is estimated at a minimum of two wavelengths of light; typically one wavelength is close to that point at which the absorption spectra of oxyhaemoglobin and deoxyhaemoglobin coincide (the isobestic point, about 805 nm), and another wavelength is at a point where the two differ markedly. From these values it is possible to calculate the concentration ratio of oxyhaemoglobin to deoxyhaemoglobin, and hence arrive at a value for oxygen saturation. Because this value is derived *in vivo* from pulsatile blood rather than from an arterial blood sample, it is conventionally given the symbol Sp_{O2}.

A pulse oximeter is a useful monitor, but some of its limitations should be borne in mind. First, by measuring saturation, it gives no more than an indication of the oxygen content of blood which is dependent also on the concentration of haemoglobin present (see Chapter 22). Secondly, the value of Sp_{O2} calculated by some machines is influenced by the presence of abnormal species of haemoglobin such as methaemoglobin and carboxyhaemoglobin. Thirdly, these machines are notoriously sensitive to artefact, such as patient movement, poor peripheral circulation and interference from electrosurgical apparatus.

Measurement of carbon dioxide concentration in expired gas

Currently, there is no easy way to measure the tension of CO_2 in the blood other than by taking an arterial sample.

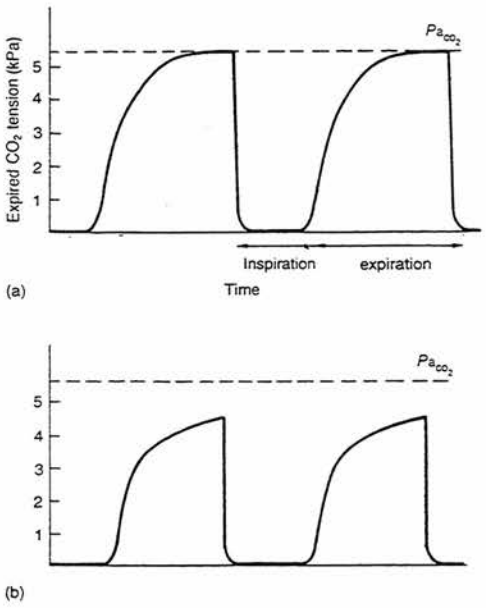

Figure 25.5 (a) Normal capnogram. (b) Capnogram in patient with chronic obstructive airways disease.

Instead, monitors are available to measure the partial pressure of CO_2 in expired air. Such monitors, called capnographs, usually rely on infrared absorption spectrometry and are able to function quickly enough to give a breath-by-breath readout of CO_2. A graph of CO_2 tension against time (a capnogram) obtained in this way is shown in Fig. 25.5a.

Measuring the expired CO_2 tension in this way is useful in a number of ways. If any capnogram is obtained, this demonstrates the integrity of the respiratory tract (excluding, for example, oesophageal intubation) and also the adequacy of the circulation. In patients on anaesthetic circuits, any CO_2 in the inspired gas suggests that the circuit is defective or is not being used properly.

In normal subjects, the *end tidal* CO_2 ($P\acute{E}_{CO2}$), that is to say the partial pressure of CO_2 in the final few millilitres of an expired breath, is representative of the average tensions of CO_2 in alveolar gas. This is in turn very close to the tension of CO_2 in the plasma as carbon dioxide is very soluble in plasma. In most people, the $P\acute{E}_{CO2}$ can be used as an estimate of the plasma CO_2 tension, ensuring the adequacy of spontaneous or artificial ventilation.

In patients with respiratory disease and a significant degree of ventilation/perfusion mismatch, the trace of expired CO_2 against time is similar to that seen in Fig. 25.5b. Instead of being nearly flat, the plateau phase of the trace rises markedly with time. This is because well ventilated alveoli with low CO_2 tensions empty early in expiration, while poorly ventilated alveoli with high CO_2 tensions tend to empty later. Such subjects typically have a large 'shunt' due to blood passing through underventilated sections of lung, and therefore have a relatively large alveolar–arterial P_{CO2} difference. For this reason the $P\acute{E}_{CO2}$ usually underestimates the arterial tension of CO_2.

Further reading

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"Respiratory Physiology"

In the Physiological Principles in Medicine Series
Published by Edward Arnold
1st Edition 1983. 2nd Edition 1992.
With John Widdicombe.

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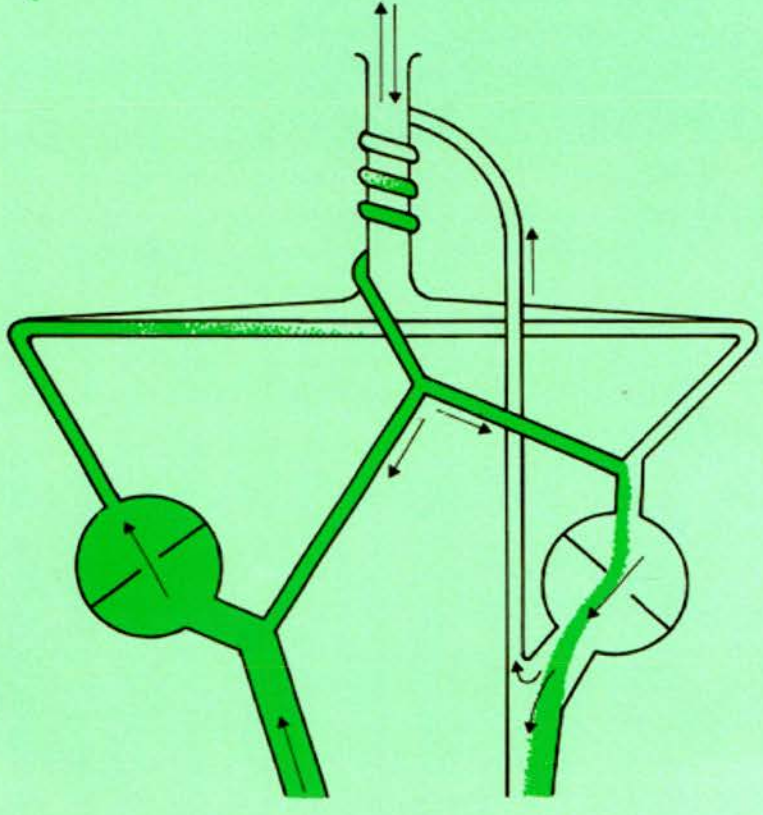
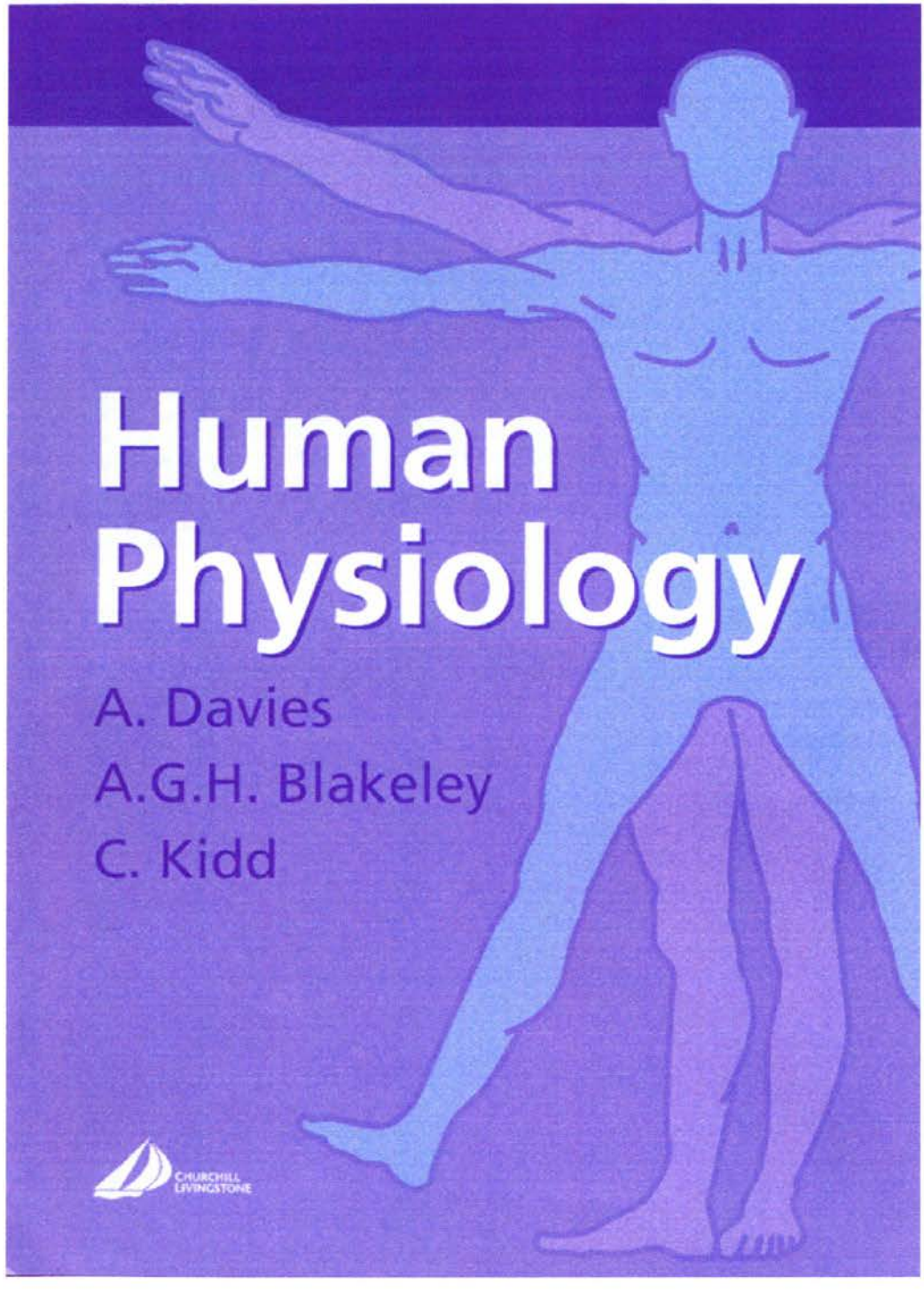


Publication 79.

"Human Physiology", (In Press)
With C.Kidd and A.Blakeley,
For Harcourt Brace(Expected September 2000)
Outline enclosed.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Publication 80.

"The Respiratory System" (In Press)
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SYSTEMS OF THE BODY

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